CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 3 · NOVEMBER, 1943 NUMBER 11

# Chemotherapeutic Studies on Transmitted Mouse Leukemia*

C. M. Flory, M.D., Ph.D., J. Furth, M.D., J. A. Saxton, Jr., M.D., and L. Reiner, M.D.

*(From the Department of Pathology of Cornell University Medical College and New York Hospital, New York, N. Y., and The Experimental Research Laboratories of Burroughs Wellcome & Co., Tuckahoe, N. Y.)*

(Received for publication June 3, 1943)

Transmitted mouse leukemia offers a good means of investigating the therapeutic action of chemical and physical agents on leukemic cells. There are few reports on the experimental chemotherapy of leukemia in mammals other than man, except for those on the effect of radioactive substances (8, 9, 11). Therapeutic studies on fowl leukosis are not directly applicable to mammals because leukemia of chickens is caused by a virus that is more resistant to physical agents than are mammalian leukemic cells (13).

Lits, Kirschbaum, and Strong (20) have studied the action of colchicine on grafted malignant lymphoid tumors in mice. Subcutaneous injections of this drug caused transient diminution in the size of tumors, but the fatal termination of the induced leukemia was not prevented. Fourteen treated animals survived an average of 50.5 days, the 13 controls 31.5 days. If treatment was delayed for 23 days, when leukemia had already become generalized, colchicine hastened death.

Forkner (7) has fully reviewed the large number of therapeutic agents used in the treatment of human leukemia. Various forms of irradiation, potassium arsenite, usually given in the form of Fowler's solution, and benzene have been most widely used.

In seeking chemicals that might serve as a nucleus in the search for therapeutic agents, the following assumptions were made:

Arsenicals and benzene have a beneficial effect on human leukemia. Both chemicals, as well as x-rays, another agent that may prolong the course of leukemia, have a depressive action on blood-forming tissues. It is generally believed that neoplastic blood cells or blood-forming tissues are more susceptible to such agents than normal cells or tissues. It would seem profitable, therefore, to investigate the action on experimental leukemia of these and other chemicals known to depress normal blood-forming organs.

Leukemia is now considered by most investigators to be a neoplastic disease. Physical, chemical, and other agents that affect other types of neoplasms may also favorably influence the course of leukemia.

Large numbers of chemicals have been used in attempts to treat human and experimental cancers; a review of these is beyond the scope of this work. Most of these substances have fallen into disrepute, but several recent studies are encouraging. Strong (29) produced permanent regression in 9 of 50 spontaneous mammary carcinomas in mice that were given a diet containing heptyl aldehyde with methyl salicylate. Boyland and Mawson (4) found that citral caused some inhibition of growth of both transmitted sarcoma and spontaneous mammary carcinoma of mice, while hepataldehyde inhibited the spontaneous tumors only. Boyland (2) also showed that among many aromatic compounds tested on mice only 4,4′-diaminodiphenylsulfoxide and sodium sulfanilyl-sulfanilate inhibited the growth of transplanted sarcoma and of spontaneous mammary tumors. In 3 of 4 mice the spontaneous mammary tumors regressed completely. Muscle extracts have been shown to inhibit the growth of mammary tumors. Boyland (3) found that this inhibiting action was contained in the aliphatic bases. Though he did not isolate a particular compound responsible for this action, he tested several compounds that might be expected to occur in such muscle extracts and found ethanolamine and cadaverine to have the greatest inhibiting action.

All chemotherapeutic studies must be evaluated in

* These investigations have been supported by The Jane Coffin Childs Memorial Fund for Medical Research, The International Cancer Research Foundation, the Lady Tata Memorial Trust, and The Anna Fuller Fund.

the light of recent investigations on the effect of underfeeding on neoplastic diseases. Sugiura and Benedict (30) have reviewed the effect of underfeeding on transmitted tumors and have shown that this treatment slows the growth of transplants and prolongs the lives of animals with such neoplasms. Tannenbaum (31, 32); McCay, Ellis, Barnes, Smith, and Sperling (23); and Saxton (26) have shown that the incidence of spontaneous neoplasms is decreased as a consequence of retarded development brought about by qualitatively adequate but quantitatively inadequate diets. Experiments made in this laboratory (27) have shown this to be true for spontaneous mouse leukemia. Since many of the chemicals used in therapeutic experiments cause definite weight loss, their beneficial action may be indirect and analogous to that of underfeeding. Consequently, the effects of underfeeding on transmitted leukemia have been studied in the present experiments.

## MATERIAL AND METHODS

The inbred mice used were either of the Ak stock or their first generation hybrids. After intravenous inoculation with leukemic cells almost all the mice died of leukemia. In a given experiment all mice were of the same strain, from 6 to 8 weeks of age, and weaned within a week or 10 days of each other. All groups used in a single experiment were similar in weight.

The following strains of both lymphoid and myeloid leukemia were used:

*Myeloid strain 106 (Akh 106).*—This strain has been used in other experiments (1). Originally these cells had specific purple granules characteristic of promyelocytes. In the course of these studies the malignant cells have become undifferentiated and free from granules. The incubation period and duration of illness of this leukemia likewise decreased during the transfers made in the course of the experiments.

*Myeloid leukemia 1712.*—This originated in an 11 month old Ak mouse that had been irradiated at 6 months with 235 r. The malignant cell of this leukemia is a promyelocyte with scattered, round, basophilic granules in the cytoplasm and a large spherical or slightly indented nucleus. The granules give the oxidase reaction.

*Chloroleukemia 1394.*—This was derived from a mouse of the Ak stock that had been irradiated at the age of 6 months with 235 r. Ten months later the spleen, lymph nodes, and liver of the animal were greatly enlarged. All lymph nodes were light green. The blood-forming organs were heavily infiltrated by myeloblasts and oxidase-positive promyelocytes. The disease, produced by transmission, was invariably chronic, although the virulence of the strain was moderately enhanced by successive passages.

*Lymphoid leukemia 5.*—This lymphoid strain originated in a nonirradiated mouse of the Ak stock and was isolated in 1936 (10). The malignant cells were large, basophilic lymphocytes. From time to time this strain was kept in the frozen state. The disease produced by it was moderately acute even when the cells were injected in very dilute suspensions. This strain was used in the early experiments, but because of the rapid course of illness it seemed advisable to isolate more chronic strains.

*Lymphoid leukemias 18, 166, and 1032.*—All these strains were isolated in the course of these studies from leukemias occurring spontaneously in mice of the Ak stock. At first these strains were subacute, but in the course of successive passages the illness produced by them became more acute. Their predominant cells were histologically similar to large lymphocytes.

*Lymphoid leukemia 1630.*—This strain was recently isolated from a spontaneous leukemia in a mouse of the Ak stock. The malignant cells were like large lymphocytes with the ability to infiltrate the liver and all blood-forming organs, although they were not found in the circulating blood in conspicuous numbers. The course of the disease was subacute or chronic.

The diseases produced by these strains of leukemia in mice were similar, both microscopically and in the gross, to the myeloid leukemias, the myeloid chloroleukemia, and the lymphoid leukemias that have been described previously (1, 14, 12).

Cell suspensions for inoculation were obtained by mincing leukemic spleen and lymph nodes in chilled Tyrode's solution and filtering out the coarse material through a cotton pledget. One-tenth cubic centimeter of the suspension or a dilution of the suspension in Tyrode's solution was injected into the tail vein of mice. From 5,000 to 5,000,000 malignant cells were thus inoculated, depending on the virulence of the strain. After all the mice in an experiment had been inoculated they were divided into comparable groups for therapy.

After death all mice were autopsied, and histological sections made whenever the diagnosis was in doubt. Mice dying of toxicity of the drugs before leukemia was detectable were not included in the tables, but are mentioned in the text.

The oral administrations were performed by dissolving the chemicals in 0.05 cc. of water or cottonseed oil and passing a curved blunt needle attached to a 0.25 cc. syringe into the esophagus as described by Morris and Thompson (24).

## EXPERIMENTAL

In the following ten tables the results of the individual experiments are summarized according to the strains of leukemia used.

In Tables I to IV appear the experiments made with myeloid leukemia 106, in Table V those with myeloid chloroleukemia 1394, and in Table VI with myeloid leukemia 1712. In Tables VII and VIII are those made with lymphoid leukemia 5, in Table IX with lymphoid leukemia 1630, and in Table X with lymphoid leukemias 18, 166, and 1032. All experiments are surveyed in Fig. 1.

TABLE I: EFFECT OF POTASSIUM ARSENITE ON MYELOID LEUKEMIA 106

| Method of administration | Experiment 1 | | | | Experiment 2 | | | | Experiment 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 4 | 4 | 17–27 | 23.0 | 10 | 10 | 12–24 | 15.2 | 6 | 6 | 16–26 | 21.7 |
| 0.2 mgm. arsenite orally 3 times weekly begun on 2nd day | 5 | 4 | 23–49 | 33.7 | | | | | | | | |
| 0.2 mgm. arsenite s.c. 3 times weekly begun on 2nd day | 5 | 4 | 36–57 | 46.0 | | | | | | | | |
| 0.1 mgm. arsenite s.c. 3 times weekly begun on 2nd day | | | | | 4 | 4 | 15–20 | 17.7 | 2 | 2 | 33&39 | 36.0 |
| 0.1 mgm. arsenite s.c. 3 times weekly begun on 5th day | | | | | 5 | 5 | 15–27 | 19.0 | | | | |

*Abbreviations.*—i.v. = Intravenous.
s.c. = Subcutaneous.
No. inj. = Number of mice injected with leukemic cells.
No. † = " " " that died of leukemia.

TABLE II: EFFECT OF BENZENE ON MYELOID LEUKEMIA 106

| Method of administration | Experiment 4 | | | | Experiment 5 | | | | Experiment 6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 6 | 6 | 16–25 | 20.0 | 4 | 4 | 14–19 | 16.0 | 9 | 9 | 13–17 | 14.6 |
| 50 mgm. benzene s.c. from 2nd to 6th days | 5 | 5 | 20–30 | 25.2 | | | | | | | | |
| 25 mgm. benzene orally from 2nd to 6th days | 6 | 4 | 25–45 | 31.0 | 5 | 2 | 21&32 | 26.5 | | | | |
| 25 mgm. benzene s.c. from 2nd to 6th days | | | | | 5 | 5 | 21–24 | 22.4 | | | | |
| 25 mgm. benzene orally from 6th to 10th days | | | | | | | | | 4 | 4 | 17–41 | 24.7 |
| 10 mgm. benzene orally from 6th to 10th days | | | | | | | | | 4 | 2 | 20&21 | 20.5 |
| 5 mgm. benzene orally 6 times weekly begun on 2nd day | | | | | | | | | | | | |

| Method of administration | Experiment 7 | | | | Experiment 8 | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 6 | 6 | 13 | 13 | 6 | 6 | 11–13 | 11.5 |
| 50 mgm. benzene s.c. from 2nd to 6th days | | | | | | | | |
| 25 mgm. benzene orally from 2nd to 6th days | 6 | 6 | 18–31 | 25.6 | 5 | 5 | 12–15 | 13.0 |
| 25 mgm. benzene s.c. from 2nd to 6th days | | | | | | | | |
| 25 mgm. benzene orally from 6th to 10th days | 6 | 6 | 18–27 | 22.6 | 5 | 4 | 11–16 | 13.5 |
| 10 mgm. benzene orally from 6th to 10th days | | | | | | | | |
| 5 mgm. benzene orally 6 times weekly begun on 2nd day | 5 | 4 | 26–32 | 29.5 | 6 | 6 | 11–14 | 12.0 |
| 5 mgm. benzene orally 6 times weekly begun on 6th day | 6 | 6 | 25–32 | 27.6 | 6 | 6 | 11–12 | 11.5 |
| 1 mgm. benzene orally 6 times weekly begun on 2nd day | 6 | 6 | 15–23 | 18.8 | | | | |

Experiments 6 and 7 were performed as a class experiment in Pathology by Miss K. M. Spellman, Mr. G. M. Walker, and Mr. L. R. Prouty.

TABLE III: EFFECT OF UNDERFEEDING AND OF THE ADMINISTRATION OF ARSENITE, MAPHARSEN, XYLENE, PSEUDOCUMENE, TRYPARSAMIDE, AND NEOARSPHENAMINE ON MYELOID LEUKEMIA 106

| Method of administration | Experiment 9 | | | | Experiment 10 | | | | Experiment 11 | | | | Experiment 12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 6 | 6 | 14–15 | 14.8 | 6 | 6 | 10–12 | 10.6 | 5 | 5 | 11–18 | 13.0 | 6 | 6 | 15–18 | 16.0 |
| Cystine 20 mgm. orally 6 times weekly begun on 2nd day | | | | | 5 | 5 | 10–13 | 11.6 | | | | | | | | |
| Underfeeding begun on 2nd day | 5 | 5 | 21–23 | 22.0 | 5 | 5 | 13–18 | 14.6 | 5 | 5 | 14–20 | 17.0 | 5 | 5 | 26–39 | 28.8 |
| Underfeeding + 20 mgm. cystine orally 6 times weekly begun on 2nd day | 5 | 5 | 26–33 | 28.4 | 5 | 5 | 12–16 | 14.0 | 4 | 4 | 15–16 | 15.7 | | | | |
| Arsenite 0.1 mgm. s.c. 6 times weekly begun on 2nd day | 5 | 5 | 19–38 | 31.2 | 6 | 6 | 11–17 | 14.0 | | | | | | | | |
| Mapharsen 0.25 mgm. s.c. 3 times weekly begun on 2nd day | 6 | 6 | 15–20 | 16.8 | | | | | | | | | | | | |
| Tryparsamide 20 mgm. s.c. 3 times weekly begun on 2nd day | | | | | | | | | 5 | 5 | 11–11 | 11.0 | | | | |
| Neoarsphenamine | | | | | | | | | | | | | | | | |
| 2 mgm. i.v. 3 times weekly begun on 2nd day | | | | | | | | | | | | | 5 | 5 | 16–17 | 16.3 |
| 2 mgm. i.v. 3 " " " " 6th " | | | | | | | | | | | | | 4 | 4 | 17–23 | 19.5 |
| 0.5 mgm. i.v. 3 times weekly begun on 2nd day | | | | | | | | | | | | | 6 | 6 | 14–16 | 15.2 |
| 0.5 mgm. i.v. 3 " " " " 6th " | | | | | | | | | | | | | 6 | 6 | 14–16 | 14.7 |
| Xylene 25 mgm. orally 6 times weekly begun on 2nd day | 5 | 5 | 12–17 | 15.0 | | | | | | | | | | | | |
| Pseudocumene 25 mgm. orally 6 times weekly begun on 2nd day | | | | | | | | | 5 | 5 | 10–16 | 12.4 | | | | |

TABLE IV: EFFECT OF SULFADIAZINE AND SULFAGUANIDINE ON MYELOID LEUKEMIA 106

| Method of administration | Experiment 13 | | | | Experiment 14 | | | | Experiment 15 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 6 | 6 | 15 | 15 | 4 | 4 | 16–19 | 17.5 | 5 | 5 | 14–16 | 14.6 |
| Sulfadiazine 20 mgm. orally 6 times weekly begun on 2nd day | 6 | 6 | 18–26 | 20.8 | | | | | 3 | 3 | 15 | 15 |
| Sulfadiazine 40 mgm. orally 6 times weekly begun on 2nd day | | | | | 4 | 4 | 17–24 | 20.5 | | | | |
| Sulfaguanidine 100 mgm. orally 6 times weekly begun on 2nd day | | | | | 5 | 5 | 16–21 | 18.2 | | | | |
| Sulfaguanidine 50 mgm. orally 6 times weekly begun on 2nd day | 6 | 6 | 16–21 | 18.7 | | | | | 5 | 5 | 13–17 | 16.0 |

Several additional experiments were done with myeloid leukemia 106. In the first (experiment 16), toluene, given orally in doses of 10 and 25 mgm. 6 times a week from the second day after inoculation, did not prolong the average lives of the treated animals (16.8 and 14.8 days respectively) over those of the controls (15.8 days). Underfed mice survived 18.6 days.

In experiment 17 the control mice lived an average of 11.2 days, while those given 25 mgm. toluene, as in the experiment reported above, survived 12.0 days, those given 25 mgm. xylene 11.6 days, and the underfed mice 13.6 days.

Pseudocumene was used in experiment 18. The control mice died in 13.0 days, while those given 1 mgm. of the drug orally 6 times a week from the second day after the inoculation survived 17.0 days; those given 10 mgm., 16.2 days; and those given 25 mgm. from the second to sixth days only, 14.8 days.

In experiment 19 with this strain of leukemia stilbestrol, given orally or intramuscularly in doses of 1 mgm. 6 times a week from the second day after inoculation, slightly shortened the average lives of the treated animals (20.5 days) as compared with those of the controls (25.0 days). In experiment 20, 0.25 mgm. of this drug given as in the previous experiment failed to prolong the average lives of the treated animals (18.5 days) over that of the controls (17.3 days).

TABLE V: EFFECT OF UNDERFEEDING AND OF THE ADMINISTRATION OF ARSENITE, BENZENE, PSEUDOCUMENE, AND X-RAY ON CHLOROLEUKEMIA 1394

| Method of administration | Experiment 21 | | | | Experiment 22 | | | | Experiment 23 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 6 | 6 | 37–58 | 51.0 | 9 | 9 | 28–42 | 34.0 | 7 | 7 | 19–28 | 22.5 |
| Underfeeding begun on 2nd day | 6 | 6 | 47–75 | 64.2 | 10 | 10 | 22–57 | 44.8 | 8 | 8 | 20–34 | 25.6 |
| Arsenite 0.1 mgm. s.c. 6 times weekly begun on 2nd day | 6 | 6 | 38–58 | 47.6 | 6 | 6 | 22–33 | 27.6 | | | | |
| Benzene 5 mgm. orally 6 times weekly: | | | | | | | | | | | | |
| begun on 2nd day | 6 | 6 | 106–148 | 127.0 | 9 | 7 | 90–? (2 alive 152 days) | 121+ | 6 | 3 | 89–? (3 alive 120 days) | 109+ |
| begun on 8th day | | | | | | | | | 7 | 7 | 45–113 | 69.0 |
| begun on 15th day | | | | | | | | | 7 | 7 | 18–68 | 34.4 |
| begun on 19th day | | | | | | | | | 3* | 3 | 23–82 | 56.0 |
| Pseudocumene 1 mgm. orally 6 times weekly begun on 2nd day | 6 | 6 | 28–57 | 48.5 | | | | | | | | |
| X-ray, 203 r on 2nd, 23rd days; 200 r on 34th day | 4 | 4 | 61–76 | 70.8 | | | | | | | | |
| 203 r on 2nd, 18th, 34th days | | | | | 9 | 9 | 43–56 | 49.0 | | | | |

* An additional 3 mice of this group died on the 18th and 19th days, before treatment had begun, and 1 died within 24 hours after the onset of treatment.

In experiment 24 with chloroleukemia 1394 the following substances were tested: 10 and 2 mgm. of ethylbenzene, 5 and 1 mgm. of cymene, and 10 mgm. of *o*-xylene each given orally 6 times a week beginning on the second day, and 1 mgm. soamine given 3 times a week subcutaneously. No prolongation of life was obtained with any of the substances tested.

TABLE VI: EFFECT OF UNDERFEEDING AND OF THE ADMINISTRATION OF ARSENITE, BENZENE, PSEUDOCUMENE, AND X-RAYS ON MYELOID LEUKEMIA 1712

| Method of administration | Experiment 25 | | | | Experiment 26 | | | | Experiment 27 | | | | Experiment 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 5 | 5 | 41–98 | 56.4 | 6 | 6 | 16–26 | 21.3 | 6 | 6 | 19–57 | 29.8 | 6 | 6 | 23–37 | 29.1 |
| Underfeeding begun on 2nd day | | | | | 6 | 6 | 20–25 | 22.0 | 5 | 5 | 19–32 | 24.5 | 6 | 6 | 22–31 | 26.5 |
| Potassium arsenite 0.1 mgm. s.c. 6 times weekly begun on 2nd day | 4 | 4 | 46–64 | 56.7 | 5 | 5 | 18–27 | 19.2 | 6 | 6 | 17–29 | 20.6 | 5 | 5 | 20–29 | 24.0 |
| Benzene 5 mgm. orally 6 times weekly begun on 2nd day | | | | | 6 | 6 | 18–24 | 20.0 | 5 | 5 | 22–36 | 27.2 | 6 | 6 | 18–30 | 24.8 |
| Pseudocumene 1 mgm. orally 6 times weekly begun on 2nd day | | | | | 6 | 6 | 18–20 | 18.2 | 6 | 6 | 20–29 | 23.0 | | | | |
| X-ray, 203 r on 2nd, 13th, 19th days | | | | | 6 | 6 | 25 | 25.0 | | | | | | | | |
| 203 r on 2nd, 11th days | | | | | | | | | 5 | 5 | 18–41 | 33.6 | | | | |
| 203 r on 4th, 17th days | | | | | | | | | | | | | 6 | 6 | 24–38 | 30.3 |

TABLE VII: EFFECT OF UNDERFEEDING AND OF THE ADMINISTRATION OF ARSENITE, NEOARSPHENAMINE, TRYPARSAMIDE, AND PSEUDOCUMENE ON LYMPHOID LEUKEMIA 5

| Method of administration | Experiment 29 | | | | Experiment 30 | | | | Experiment 31 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Control | 9 | 9 | 11–22 | 15.4 | 5 | 5 | 10–13 | 11.0 | 5 | 5 | 11–16 | 12.6 |
| Underfeeding begun on 2nd day | 11 | 11 | 13–18 | 14.7 | 6 | 6 | 12–16 | 14.0 | | | | |
| Arsenite 0.1 mgm. 3 times weekly begun on 2nd day | 6 | 6 | 13–16 | 14.5 | 4 | 4 | 11–12 | 11.3 | | | | |
| Arsenite 0.1 mgm. 3 times weekly begun on 6th day | 6 | 6 | 12–14 | 13.1 | | | | | | | | |
| Arsenite 0.1 mgm. 6 times weekly begun on 2nd day | | | | | 5 | 5 | 11–12 | 11.2 | | | | |
| Neoarsphenamine 2 mgm. 6 times weekly begun on 2nd day | | | | | 6 | 6 | 11–13 | 12.0 | | | | |
| Tryparsamide 20 mgm. 3 times weekly begun on 2nd day | | | | | | | | | 4 | 4 | 13–23 | 17.2 |
| Pseudocumene 25 mgm. 6 times weekly begun on 2nd day | | | | | | | | | 6 | 6 | 10–15 | 11.2 |

TABLE VIII: EFFECT OF UNDERFEEDING AND OF THE ADMINISTRATION OF BENZENE, TOLUENE, XYLENE, SULFADIAZINE, AND SULFAGUANIDINE ON LYMPHOID LEUKEMIA 5

| Method of administration | Experiment 32 | | | | Experiment 33 | | | | Experiment 34 | | | | Experiment 35 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 4 | 4 | 9–11 | 10.0 | 7 | 7 | 11–16 | 12.5 | 5 | 5 | 10–12 | 11.2 | 4 | 4 | 9–11 | 10.0 |
| Underfeeding begun on 2nd day | | | | | 6 | 6 | 15–19 | 17.2 | 5 | 5 | 10–12 | 11.2 | | | | |
| Benzene 25 mgm. orally from 2nd to 6th day | 4 | 4 | 9–24 | 15.5 | 6 | 6 | 13–19 | 16.8 | | | | | 4 | 4 | 9–24 | 15.7 |
| Benzene 25 mgm. s.c. from 2nd to 6th day | 4 | 4 | 12–17 | 13.0 | | | | | | | | | 4 | 4 | 12–17 | 13.7 |
| Benzene 25 mgm. orally from 6th to 10th day | | | | | 6 | 6 | 12–25 | 15.5 | | | | | | | | |
| Benzene 10 mgm. orally from 2nd to 9th day | | | | | 6 | 6 | 13–20 | 17.0 | | | | | | | | |
| Benzene 10 mgm. orally from 6th to 13th day | | | | | 6 | 6 | 13–17 | 14.0 | | | | | | | | |
| Toluene 25 mgm. orally from 2nd to 8th day | | | | | | | | | 4 | 4 | 10–14 | 12.0 | | | | |
| Xylene 25 mgm. orally from 2nd to 8th day | | | | | | | | | 5 | 5 | 10–14 | 11.6 | | | | |
| Sulfadiazine 40 mgm. orally 6 times weekly begun on 2nd day | | | | | | | | | | | | | 4 | 4 | 10–15 | 13.0 |
| Sulfaguanidine 100 mgm. orally 6 times weekly begun on 2nd day | | | | | | | | | | | | | 5 | 5 | 9–22 | 14.8 |

TABLE IX: EFFECT OF ARSENITE, BENZENE, ETHYLBENZENE, CYMENE, PSEUDOCUMENE, AND X-RAY ON LYMPHOID LEUKEMIA 1630

| Method of administration | Experiment 36 | | | | Experiment 37 | | | | Experiment 38 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 6 | 6 | 23–24 | 23.9 | 6 | 6 | 23–40 | 31.2 | 5 | 5 | 19–25 | 20.7 |
| Arsenite 0.1 mgm. s.c. 6 times weekly begun on 2nd day | 6 | 6 | 22–26 | 24.0 | | | | | 6 | 6 | 17–25 | 20.7 |
| Benzene 2 mgm. orally 6 times weekly begun on 3rd day | 6 | 6 | 23–26 | 24.0 | | | | | | | | |
| Ethylbenzene 25 mgm. orally 6 times weekly begun on 5th day | | | | | 6 | 6 | 12–37 | 22.7 | | | | |
| Ethylbenzene 5 mgm. orally 6 times weekly begun on 5th day | | | | | 6 | 6 | 18–29 | 24.3 | | | | |
| Ethylbenzene 1 mgm. orally 6 times weekly begun on 2nd day | | | | | | | | | 6 | 6 | 17–21 | 19.0 |
| Cymene 25 mgm. orally 6 times weekly begun on 5th day | | | | | 2 | 2 | 20&27 | 23.5 | | | | |
| Cymene 5 mgm. orally 6 times weekly begun on 5th day | | | | | 6 | 6 | 20–26 | 23.2 | | | | |
| Cymene 1 mgm. orally 6 times weekly begun on 2nd day | | | | | | | | | 6 | 6 | 17–21 | 19.3 |
| Pseudocumene 1 mgm. orally 6 times weekly begun on 2nd day | 6 | 6 | 9–33 | 23.2 | | | | | | | | |
| X-ray 203 r on 3rd and 9th days | 6 | 6 | 22–42 | 26.8 | | | | | | | | |

TABLE X: EFFECT OF ARSENITE, SOAMINE, BENZENE, XYLENES, PSEUDOCUMENE, AND MESITYLENE ON LYMPHOID LEUKEMIAS 18, 166, AND 1032

| Method of administration | *Strain 18* Experiment 39 | | | | *Strain 166* Experiment 40 | | | | *Strain 1032* Experiment 41 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Survival | | | | Survival | | | | Survival | |
| | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days | No. inj. | No. † | Extreme, days | Average, days |
| Controls | 5 | 5 | 22–30 | 27.0 | 4 | 4 | 14–27 | 19.2 | 5 | 5 | 10–11 | 10.4 |
| Arsenite 0.1 mgm. s.c. 3 times weekly begun on 2nd day | | | | | | | | | 6 | 6 | 10–11 | 10.5 |
| Soamine 1 mgm. s.c. 3 times weekly begun on 2nd day | 4 | 4 | 27–29 | 28.0 | 6 | 6 | 15–27 | 19.2 | | | | |
| Benzene 25 mgm. orally from 2nd to 6th days | | | | | | | | | 5 | 5 | 12–14 | 12.8 |
| *o*-Xylene 25 mgm. orally from 2nd to 10th days | 2 | 2 | 16&27 | 21.5 | | | | | | | | |
| *p*-Xylene 25 mgm. orally from 2nd to 10th days | 4 | 4 | 15–29 | 23.5 | | | | | | | | |
| Pseudocumene 25 mgm. orally from 2nd to 10th days | | | | | 5 | 5 | 17–36 | 25.5 | | | | |
| Mesitylene 25 mgm. orally 6 times weekly begun on 2nd day | | | | | 5 | 5 | 14–21 | 15.4 | | | | |

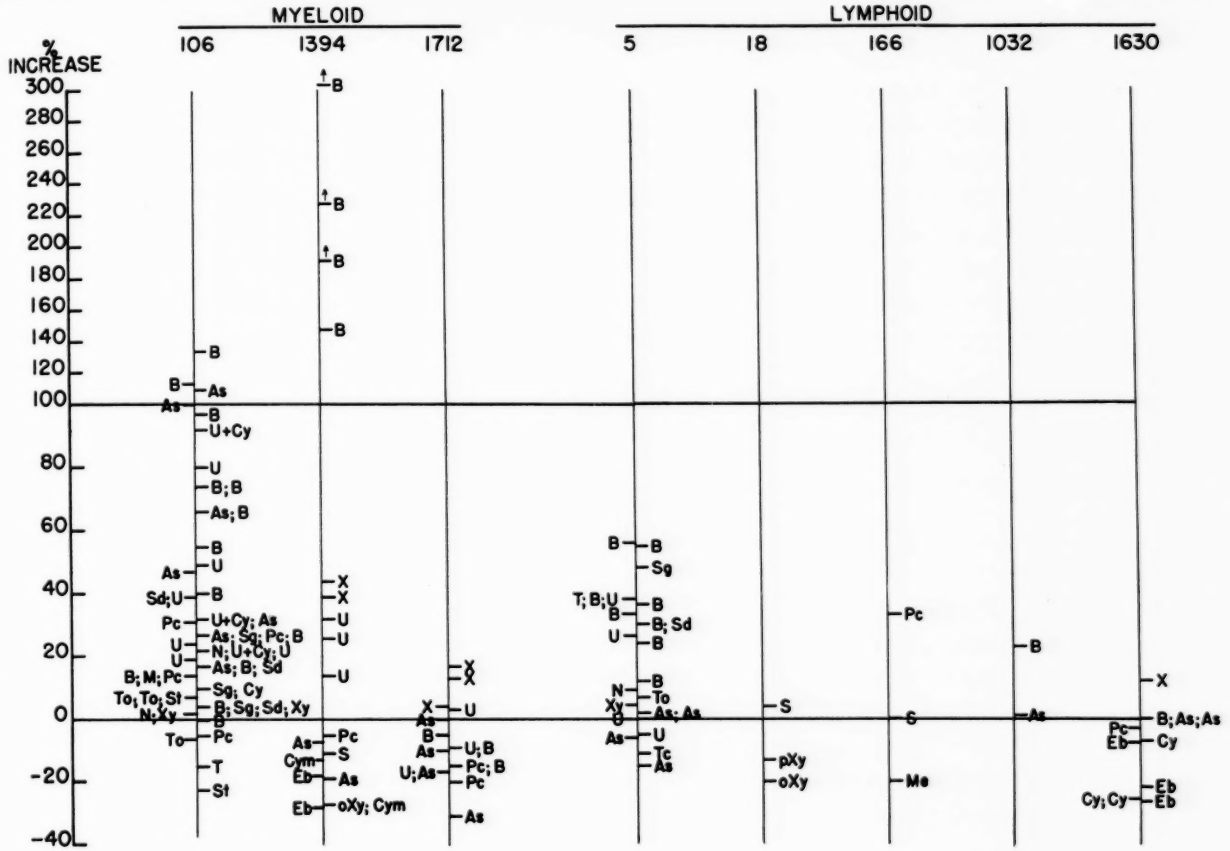



FIG. 1.—A summary of all experiments arranged according to the strain of leukemia used. The average length of life of the treated mice divided by that of the control mice is expressed as "percentage increase." The small arrow indicates that some animals in the experiment are still alive and that the final percentage increase will be greater.

*Abbreviations*

As = Potassium arsenite.
B = Benzene.
Cy = Cystine.
Cym = Cymene.
Eb = Ethylbenzene.
M = Mapharsen.
Me = Mesitylene.
N = Neoarsphenamine.
oXy = *o*-Xylene.
Pc = Pseudocumene.
pXy = *p*-Xylene.
S = Soamine.
Sd = Sulfadiazine.
Sg = Sulfaguanidine.
St = Stilbestrol.
T = Tryparsamide.
To = Toluene.
U = Underfeeding.
X = X-ray.
Xy = Commercial xylene.

## Effect of Potassium Arsenite

Toxicity: Potassium arsenite dissolved in distilled water and injected subcutaneously was tolerated by 6 week old mice in doses of 0.1 mgm. daily, and by slightly older mice in doses of 0.2 mgm. A single injection of 0.3 mgm. killed all mice.

Further tests revealed that 0.2 mgm. often killed mice, but that 0.1 mgm. could be given 6 times a week. This dosage was used in all subsequent experiments.

Therapeutic effect: In experiment 1 (Table I), made with myeloid leukemia 106, 0.2 mgm. of potassium arsenite administered subcutaneously doubled the lives of the leukemic mice, but oral administration was less effective. In experiment 2, 0.1 mgm., also given 3 times a week, only slightly prolonged life. In experiment 3, 6 mice were started on 0.2 mgm. but 4 were killed by the first inoculation. Given 0.1 mgm. the remaining 2 mice outlived the controls by about 60 per cent.

In experiment 9 (Table III) 0.1 mgm. of arsenite given 6 times a week doubled the survival time of the leukemic mice. In experiment 10 the prolongation of life was only about 20 per cent, but in this experiment the leukemia progressed very rapidly. In both these experiments underfeeding alone was as effective as arsenite.

With strain 106, then, arsenite prolonged the course of the disease in all experiments, but this effect was variable, being definite in some of the groups tested and slight in others.

In contrast to this, treatment with arsenite may have shortened life slightly with chloroleukemia 1394 (Table V, experiments 21 and 22). Myeloid leukemia 1712 (Table VI, experiments 25 to 28) was unaffected by arsenite.

None of the lymphoid leukemias tested was sensitive to arsenite. Strains 5 (Table VII, experiments 29 and 30), 1630 (Table IX, experiments 36 and 38, and 1032 (Table X) were used.

### Other Arsenicals

*Neoarsphenamine* (sodium 3,3-diamino-4,4-dihydroxyarsenobenzene methanal sulfoxylate).—Toxicity: Ten milligrams of this drug dissolved in water and injected intravenously killed all mice, but 2 mgm. was tolerated when given 3 times a week.

Therapeutic effect: No significant effect was obtained on myeloid leukemia 106 (Table III, experiment 12) or on lymphoid leukemia 5 (Table VII, experiment 30). This myeloid strain responded moderately well to arsenite but was not tested in the same experiment. The lymphoid strain failed to respond to arsenite.

*Mapharsen* (3-amino-4-hydroxy phenylarsine oxide).—Toxicity: One-half milligram in water injected intravenously killed over half the mice, but 0.25 mgm. given 3 times a week was tolerated.

Therapeutic effect: No effect was apparent on the myeloid strain 106 (Table III, experiment 9). In the same experiment arsenite doubled the length of life of the leukemic mice.

*Tryparsamide* (sodium N-phenylglycinamide-4-arsonate).—Toxicity: Forty milligrams injected intravenously in water killed almost all mice, but 20 mgm. given 3 times a week was tolerated.

Therapeutic effect: This drug had no effect on the length of life of the mice with myeloid leukemia 106 (Table III, experiment 11) unless to shorten it. The drug did, however, prolong the course of lymphoid leukemia 5 (Table VII, experiment 31). This finding is worthy of further investigation, since this strain did not respond to arsenite.

*Soamine* (sodium 4-aminophenylarsonate, atoxyl).—Toxicity: Two milligrams dissolved in water and given intravenously killed mice after 3 or 4 doses, but 1 mgm. given 3 times a week was well tolerated.

Therapeutic effect: No effect was obtained on lymphoid leukemias 18 and 166 (Table X) and on chloroleukemia 1394 (experiment 24). These lymphoid leukemias have not been tested concurrently with other chemicals. The chloroleukemia responded well to benzene but not to arsenite.

### Effect of Benzene and Related Compounds

*Benzene.*—Toxicity: The toxicity of benzene was difficult to determine because of its delayed and chronic effects. Fifty milligrams given orally on 4 consecutive days often killed the mice, but 25 mgm. given for the same length of time was tolerated. Ten milligrams was not toxic if given on 6 or 7 consecutive days. Five milligrams given 6 times a week was tolerated for over 100 days. All doses of benzene were given in cottonseed oil, usually in a volume of 0.05 cc.

Occasional periods of 5 or 6 days during which no benzene was given seemed to benefit the mice when prolonged treatment was necessary, as with chloroleukemia 1394 (Table V).

Therapeutic effect: This drug distinctly prolonged the course of myeloid leukemia 106 (Table II, experiments 4 to 7). Twenty-five milligrams given from the second to the sixth day prolonged the lives of the leukemic mice from 50 to almost 100 per cent. Five milligrams daily was even more effective, but 1 mgm. daily had only a slight effect (experiment 7). Only in experiment 8 (Table II) did benzene have little effect. In this experiment the leukemia was very acute, and this may account for the failure.

Benzene had, however, no effect on myeloid leukemia 1712 (Table VI, experiments 26 to 28).

The third myeloid strain, chloroleukemia 1394, was highly susceptible to treatment with benzene. In experiment 21 (Table V) the mice treated with 5 mgm. of benzene 6 times a week survived twice as long as the controls, and in experiment 22 over 3 times as long. In both experiments x-ray and underfeeding prolonged life, though not as much as benzene, while arsenite may have shortened it.

The effect of the administration of benzene to mice with well advanced chloroleukemia 1394 was determined in experiment 23 (Table V). The untreated leukemic animals survived from 19 to 28 days, an average of 22.5 days. When therapy with this chemical was begun on the day after the inoculation of the leukemic cells, the mice lived the longest, an average of 109 days, or about 5 times as long as the untreated mice. When treatment was begun on the eighth day the mice survived for an average of 69 days, but even when the administration of benzene was delayed until the 15th or 19th day some effect was obtained. Of the 7 mice in which the therapy was begun on the 15th day, one died on the 18th, 2 on the 19th, and one each on the 25th and 26th days. These 5 survived only as long as the controls, but the remaining 2 lived for 66 and 68 days, or 3 times as long as the controls. A second group of 7 mice was scheduled to be placed on benzene therapy on the 22nd day, but on the 18th day one mouse died of leukemia, and 2 on the 19th. Nevertheless, therapy was started on the remaining 4 mice. These died after 20, 23, 63, and 82 days. This would indicate that treatment with benzene may prolong the lives of mice even when leukemia is advanced. The effect of benzene on this chloroleukemia was the most

beneficial of any substance tested in this series of experiments.

On lymphoid leukemias benzene was slightly or moderately effective. In experiments 32 and 35 (Table VIII) this drug prolonged the lives of the mice with strain 5 leukemia by a maximum of about 50 per cent. In experiment 33 the effect was less definite, and the underfed mice survived longest, suggesting that the effect of benzene on this strain was a generalized toxic effect rather than a specific effect. On strain 1630 (Table IX, experiment 36) benzene had no effect, but the dose used was rather small (2 mgm.). Benzene had only a slight effect on lymphoid leukemia 1032 (Table X).

Thus benzene had a definite effect on 2 of the 3 myeloid strains, but only a moderate effect on one of the two lymphoid strains adequately tested.

*Benzene derivatives* (alkylbenzenes).—Toxicity and mode of administration: None of the benzene derivatives enumerated below was very toxic. When 50 mgm. of one of these liquids was given orally the mice frequently aspirated the material and died. If, however, the substance was dissolved in equal parts of cottonseed oil it could be given orally with ease. Twenty-five milligrams of these substances given 6 times a week were tolerated by the mice for the duration of the experiments without evidence of toxicity.

Therapeutic effects: *Toluene* (methylbenzene).—Toluene was used in two experiments with myeloid leukemia 106 (experiments 16 and 17). In doses of 10 and 25 mgm. daily this drug did not prolong the survival time of the treated animals over that of the controls. Toluene had no effect on lymphoid leukemia 5 (Table VIII, experiment 34).

*Xylenes* (dimethylbenzenes).—Three xylenes were used: *o*-xylene, *p*-xylene, and commercial xylene, which contains 85 per cent *m*-xylene. Myeloid leukemia 106 was not affected by 25 mgm. of commercial xylene given 6 times a week (Table III, experiment 9), although benzene and arsenite both doubled the periods of survival of the mice in the same experiment. In experiment 17, made with the same strain, commercial xylene had no effect. *o*-Xylene had no effect on chloroleukemia 1394 in experiment 24. Commercial xylene had no effect on lymphoid leukemia 5 (Table VIII, experiment 34). *o*-Xylene and *p*-xylene did not prolong the lives of mice affected with lymphoid leukemia 18 (Table X).

*Trimethylbenzenes.*—Mesitylene (1,3,5-trimethylbenzene) and pseudocumene (1,2,4-trimethylbenzene) were studied. Mesitylene was tested on lymphoid leukemia 166 (Table X) and had no effect. Pseudocumene was used more extensively. On myeloid leukemia 106 (Table III, experiment 11) no effect was obtained with 25 mgm. administered daily. In experiment 18, with this strain of leukemia, the control mice died after an average period of 13 days, while those treated with 1 mgm. survived for 17 days, those with 3 mgm. for 16.2 days, and with 25 mgm. for 14.8 days. On myeloid leukemia 1712 (Table VI, experiments 26 and 27) no effect was noted and chloroleukemia 1394 (Table V, experiment 21) also failed to respond to this drug.

No effect was seen on lymphoid leukemia 5 (Table VII, experiment 31), and on strain 1630 (Table IX, experiment 36). A slight prolongation of life was obtained with strain 166 (Table X).

*Ethylbenzene.*—This chemical had no effect on chloroleukemia 1394 (experiment 24), or on the lymphoid strain 1630 (Table IX, experiments 37 and 38).

*Cymene* (isopropyl-*p*-methylbenzene).—Chloroleukemia 1394 (experiment 24) and lymphoid leukemia 1630 (Table IX, experiments 37 and 38) were not affected by this drug.

No benzene derivatives tested approached the effectiveness of benzene itself. Some of the derivatives were, however, not tested extensively and might prove effective on certain strains of leukemia.

### Miscellaneous Compounds

*Stilbestrol* (diethylstilbestrol, 4,4′-dihydroxy-$\alpha,\beta$-diethylstilbene).—Toxicity: Four milligrams injected subcutaneously was tolerated for 10 daily doses. One milligram was given for 15 doses without killing any mice.

Therapeutic effect: Stilbestrol in daily doses of 1.0 mgm. slightly shortened the survival time of mice inoculated with myeloid leukemia 106 (experiment 19). In experiment 20, 0.25 mgm. also failed to prolong life.

*Sulfadiazine* (2-sulfanilamidopyrimidine).—Toxicity: Forty milligrams of this drug given daily with food was not toxic in 24 days, but 100 mgm. killed normal mice in 11 days.

Therapeutic effect: In experiment 13 (Table IV) 20 mgm. prolonged the lives of the mice inoculated with myeloid leukemia 106 by about one-third, but in the subsequent two experiments (14 and 15) no significant effect was obtained. The course of lymphoid leukemia 5 (Table VIII, experiment 35) was also prolonged by about one-third. This experiment was not repeated.

*Sulfaguanidine* (sulfanilylguanidine).—Toxicity: Two-hundred and fifty milligrams of this chemical given daily with food killed the mice in 8 to 14 days, but 100 mgm. was tolerated during the experiment.

Therapeutic effect: This chemical had almost no effect on myeloid leukemia 106 (Table IV, experiments 13 to 15), but slightly prolonged the course of lymphoid leukemia 5 (Table VIII, experiment 35).

*l-Cystine.*—The effect of adding *l*-cystine to the diets of underfed mice with myeloid leukemia 106 is shown in experiments 9 to 11 (Table III). In experiment 9 the underfed animals lived about 50 per cent longer than the controls, while those given supplementary *l*-cystine lived about 100 per cent longer. In experiments 10 and 11, however, the addition of *l*-cystine did not appreciably affect the lives of the underfed mice. In experiment 10 supplementary *l*-cystine also did not prolong the lives of mice on a normal diet.

### The Effect of Underfeeding

*Method of underfeeding.*—After inoculation with leukemic cells the mice were divided into groups with similar average weights. Subsequently each group was weighed once or twice a week. Underfeeding was begun on the second day. Each mouse received 1 gm. of bread wet with milk and 0.75 gm. of fox chow daily. Water was available at all times. On this quantitatively inadequate diet the mice lost a few grams and in about a week on this regime usually weighed from 16 to 17 gm. apiece. The controls maintained on fox chow continued to gain weight and usually were 7 or 8 gm. heavier than the underfed animals during most of the experiment. All mice lost 2 or 3 gm. at the advanced stage of leukemia.

*Results of underfeeding.*—In the first experiment with myeloid leukemia 106 (Table III, experiment 9) the underfed mice outlived the controls by almost 50 per cent, and the underfed animals given supplementary cystine lived about twice as long after treatment as the untreated animals. The weights of the groups of mice in this experiment are shown in Fig. 2. After the third day the underfed mice given additional *l*-cystine began to lose weight, and after the fifth day the underfed mice also began to lose weight. The arsenite-treated mice remained at 20 gm. for 18 days, their weights being the same as the mapharsen treated animals, which lived only for an average of 17 days, and slightly below those of the control mice, which died at 14.8 days.

In experiment 12 (Table III), made with the same strain of mice, the prolongation of life by underfeeding was 80 per cent. The weights of the underfed animals decreased from an average of 20 to 16 gm. in 11 days, while all other animals gained weight, that of the controls increasing from 21 to 24 gm. in the same time.

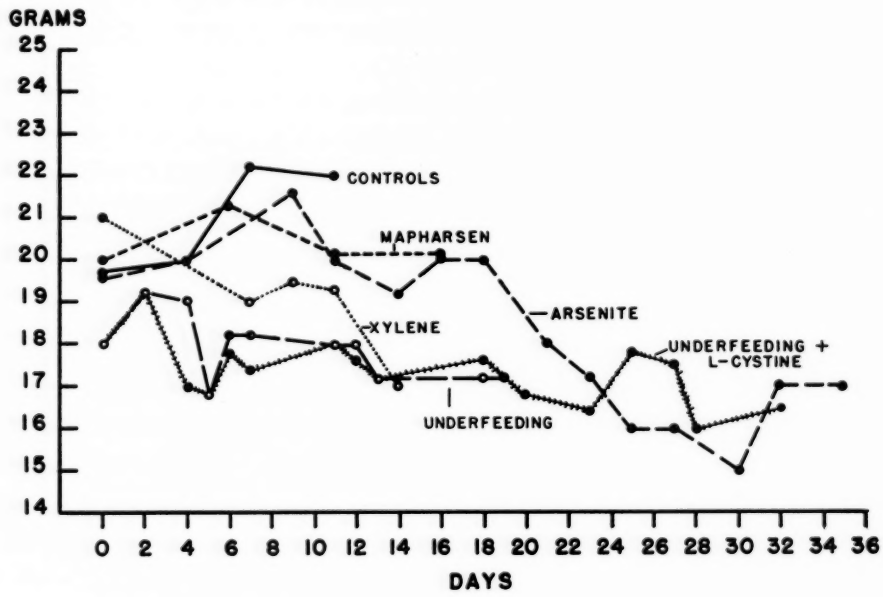



Fig. 2.—Average weights of mice in experiment 9, Table III.

In experiment 10 (Table III) the underfed animals lived as long as the arsenite-treated mice, but neither of these treatments conspicuously delayed the relatively rapid course of the leukemia. In experiment 11 (Table III) the underfed group survived the longest. In neither experiment was the prolongation of life more than about one-third. In experiment 10 the weight of the underfed mice remained at an average of 18 gm., but in experiment 11 their average weights fell from 18.4 to 15.4 gm. In experiments 16 and 17, made with the same strain, the underfed mice survived the longest, but the effect was likewise slight. Thus, on myeloid leukemia 106 underfeeding prolonged life in all experiments, the effect varying from slight to 80 per cent.

Three experiments were performed on the effect of underfeeding on myeloid strain 1712. The results are shown in Table VI. In experiment 26 the average

weights of the underfed mice fell slowly from 18 to 15.6 gm., while those of the controls did not rise over their initial weight of 17 gm. In experiment 27 the underfed mice lost weight rapidly, from 19 to 16 gm., while the controls stayed at 23 gm. In experiment 28 the weights of the underfed mice fell from 22.7 to 17 gm. (Fig. 3), while those of the other groups remained at about their original levels until leukemia set in. In none of these experiments did underfeeding prolong life.

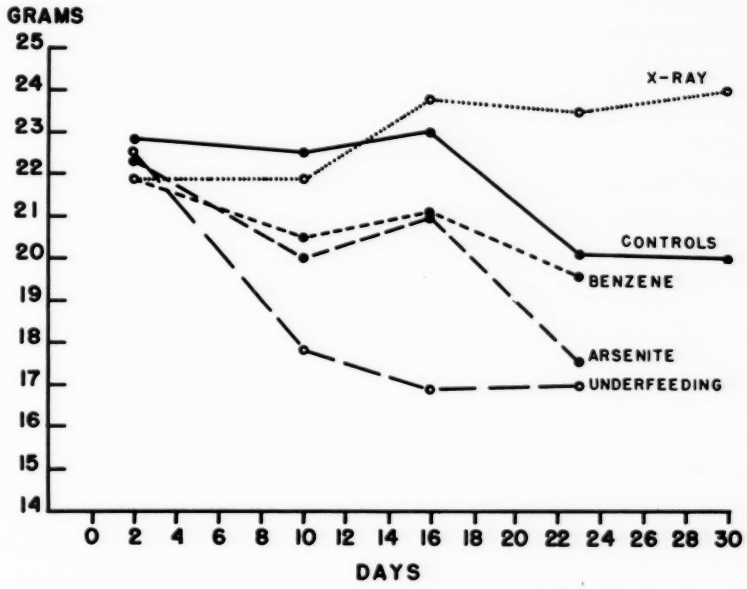



FIG. 3.—Average weights of mice in experiment 28, Table VI.

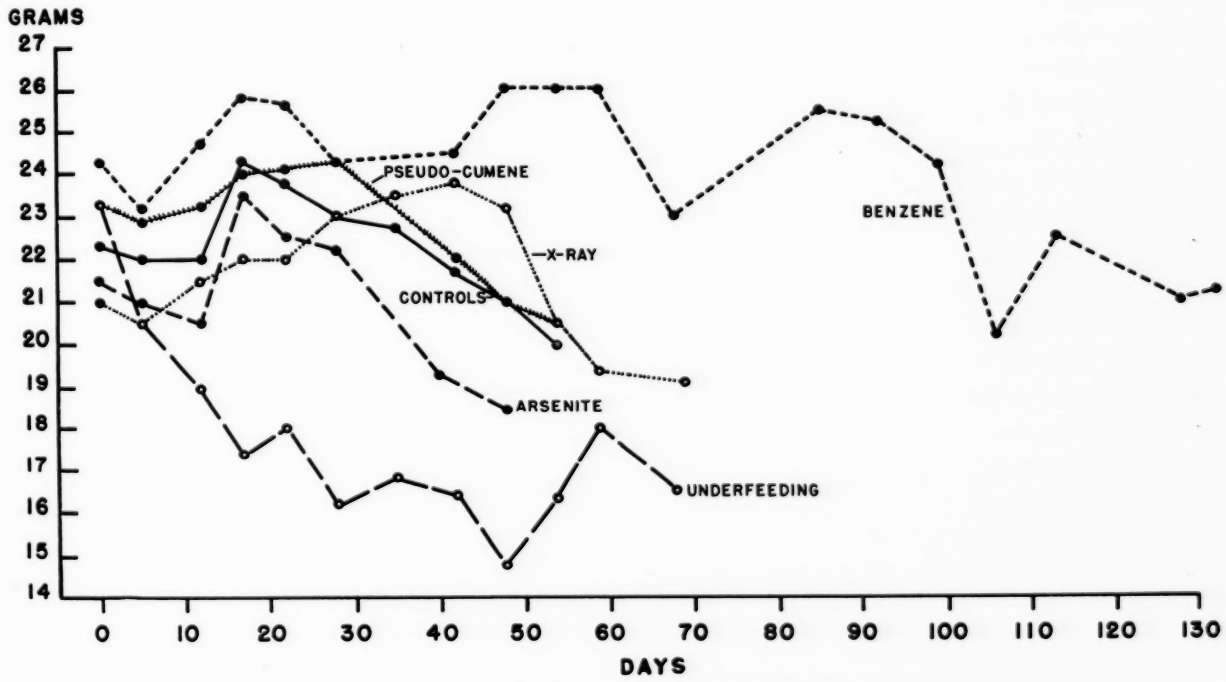



FIG. 4.—Average weights of mice in experiment 21, Table V.

In Table V the effect of underfeeding on chloroleukemia 1394 is shown. In all three experiments the underfed mice lost weight rapidly and lived slightly longer than the controls. The benzene-treated mice in these experiments, which outlived the controls and underfed mice by such a long period of time, gained weight, as did the controls. The weight curves of the mice of experiment 21 are shown in Fig. 4.

The effect of underfeeding on lymphoid leukemia 5 is shown by experiments 29 and 30 (Table VII)

and experiments 33 and 34 (Table VIII). In two of these experiments life was prolonged somewhat, but never more than 50 per cent.

### X-Rays

Previous experiments have shown that irradiation of mice inoculated with a transmitted lymphoid leukemia with x-rays or gamma rays prolonged life, but did not prevent the fatal termination of the disease (11). Subsequent work (8, 9) confirmed these findings. It was suggested that irradiation diminished the number of the injected leukemic cells in each animal, but seldom if ever destroyed all the malignant lymphocytes that had been introduced. It seemed desirable to compare the effect of x-rays with the chemicals used in these studies under comparable conditions.

The mice to be irradiated were placed in a small cage and exposed to a 140 kv., 5 ma. x-ray at 30 cm. target skin distance with no filter at the rate of 60 r per minute. The usual dose was 203 r.

With myeloid strain 1712 (Table VI) no significant prolongation of life was obtained in experiments 27 and 28. In experiment 26 the controls lived an average of 21.3 days, while all the irradiated animals died on the 25th day. This was probably the result of two doses of x-rays too closely spaced.

With chloroleukemia 1394 (Table V), x-ray caused a greater prolongation of life (39 per cent in experiment 21, and 44 per cent in experiment 22) than any agent tried except benzene.

On lymphoid leukemia 1630 (Table IX, experiment 36) x-ray had no significant effect.

## DISCUSSION

There are at the present three accepted methods of treating human leukemia. Of these, x-rays and radium are widely used throughout the world, potassium arsenite in the form of Fowler's solution in the United States, and benzene in some parts of Europe.

*X-rays and radioactive substances.*—Earlier experimental studies made with lymphoid leukemia (8, 11) have led us to suppose that x-rays and the gamma rays of radium prolong the lives of leukemic animals. Groups of mice were exposed to increasing quantities of x-rays. By single total irradiation the optimal dose was in the neighborhood of 400 to 450 r. Larger doses killed a certain number of animals, but even from these mice malignant cells could be recovered at the time of death. Fractional irradiation under the conditions tested was not more effective than single total irradiation.

Similarly, mice were subjected to uninterrupted irradiation by gamma rays of radium (9). Their lives were prolonged, but malignant cells were present even in mice killed by large doses of these rays.

In the present experiments myeloid leukemia of mice was used for the first time in therapeutic studies with x-rays. The largest dose believed safe for young mice used in these experiments was considered to be in the neighborhood of 350 r. By an unfortunate error in calculation of dosage, only 203 r was administered. The results were similar to those obtained previously with lymphoid leukemia. It is believed that irradiation usually does not benefit chronic human lymphoid leukemia as much as chronic myeloid leukemia (7). Experiments are in progress to determine if larger doses of x-rays would exert a more favorable effect on myeloid leukemias of mice.

*Arsenicals.*—Arsenic was used in the treatment of leukemia as early as 1865. It was later neglected, and was reintroduced by Claude Forkner (7). Forkner states that no case of myeloid leukemia failed to be benefited by Fowler's solution when treated early in the course of the disease. The white count, including the immature cells, was reduced, while the red count remained stationary or even increased. The size of the spleen and liver was reduced. In cases of chronic lymphoid leukemia the improvement following arsenical therapy was definitely less. Few other arsenicals have been tried on human patients. Naegeli (25) obtained "good results" with arsenious acid. Forkner, who has reviewed the subject, regards neoarsphenamine as of no value in acute leukemia (7).

The toxic action of arsenic is believed to be due to an interference with the oxidative processes governed by glutathione. Voegtlin, Dyer, and Leonard (33) suggested that arsenoxide may react chemically with the reduced form of glutathione and perhaps with some other SH compounds in the protoplasm. Maver and Voegtlin (22) subsequently showed that arsenate inhibits the initial depolymerizing activity as well as the nuclease activity of tumor and liver extracts upon thymus nucleic acid, and also the action of phosphatase. Arsenite under anaerobic conditions was less inhibitive than arsenate.

In general the trivalent arsenites are much more toxic to protozoa, bacteria, and yeast cells than the pentavalent. With intraperitoneal injection into guinea pigs the toxicity decreases in the order: arsenite, arsenates, colloidal arsenic, atoxyl, and cacodyl (28).

In our experiments one strain of myeloid leukemia (No. 1394) was affected neither by potassium arsenite nor by soamine. This strain was highly sensitive to benzene. Another myeloid strain (No. 1712) failed to respond to both potassium arsenite and benzene, while a third strain (No. 106) responded favorably to both. Other arsenicals tested (mapharsen, neoarsphenamine, and tryparsamide) were of no significant benefit to mice with myeloid leukemia 106. None of the three lymphoid strains tested responded to potassium arsenite. One of these (strain 5) responded

well to benzene, another to x-rays. In the only experiments made in which potassium arsenite was not tested, tryparsamide was moderately effective. The other arsenicals tested on lymphoid strains were neoarsphenamine and soamine, and neither of these was of any benefit.

The number of arsenicals thus far tested is small. A comparison of the therapeutic effect of pentavalent and trivalent arsenicals has not been made.

*Benzene and related compounds.*—Benzene in large amounts causes almost complete aplasia of both myeloid and lymphoid tissue. In smaller amounts, as is indicated by observation on human patients, the effects are more variable. Hunter (15) states that not only anemia but also polycythemia may be produced by benzene. In chronic benzene poisoning in human beings, Mallory, Gall, and Brickly (21) observed marrow changes ranging from severe hypoplasia to pronounced hyperplasia, and in two instances leukemia. Others have reported the occurrence of leukemia in workers exposed to benzene (34), and Lignac (18, 19) has produced leukemia in mice by its administration.

Undoubtedly this specific action on marrow cells led to the exploration of the usefulness of benzene in the control of leukemia in man. The most extensive studies of the effectiveness of this chemical in patients with leukemia were made by Korányi (17). Summarizing the results on approximately 200 patients treated with the drug, Kalapos (16) concluded that benzene is a valuable agent in the treatment of leukemia and that it reduces the size of the spleen and lymph nodes more rapidly than x-rays.

In our experiments benzene was the best of all chemicals tried. Its therapeutic index is about the same as that of arsenite. Five milligrams administered to mice daily over long periods of time proved relatively harmless.

The best results were obtained with chloroleukemia 1394. Benzene doubled or tripled the period of survival of mice with this leukemia, but it is doubtful if all leukemic cells were destroyed in any mouse. With another myeloid leukemia, strain 106, benzene was also the most beneficial agent tried, while a third, strain 1712, was refractory. While benzene is generally regarded as a specific poison to bone marrow, it was the best therapeutic agent tried for lymphoid strain 5. Unfortunately, its action in the latter experiment has not been compared with x-rays. Since these leukemic cells are preserved in the frozen state (5, 6), such supplementary experiments can still be carried out.

*Derivatives of benzene.*—These include: toluene, three xylenes, mesitylene, pseudocumene, ethylbenzene, and cymene. None of these was as effective as benzene, and some were definitely harmful. Pseudocumene seemed to be of value in lymphoid leukemia 166, but numerous other experiments, summarized in Fig. 1, discouraged further study with this compound.

*Sulfaguanidine and sulfadiazine.*—Acute leukemias in man are often accompanied by an acute infection. Our results with sulfaguanidine and sulfadiazine are of interest in this connection. These drugs failed to benefit the mice with leukemia but were otherwise harmless; thus their administration in human leukemia complicated by infection could be considered.

*Underfeeding.*—The observations made warranted the generalization that underfeeding tends to prolong the life of animals with transmitted leukemia. This effect was slight in some experiments, moderate in others. This is in accordance with the experiments of Sugiura and Benedict (30) with transmitted tumors, and raises the question whether the beneficial action that followed the administration of some of the chemicals was not secondary to retardation of the growth of the animals produced by them. This may have been true in some cases, but the very best effects by chemicals were often obtained without significant weight loss of the animals. As an example, Fig. 4 is shown (Table V, experiment 21). In this experiment benzene, the most effective chemical, did not cause a loss in weight. The control and arsenite-treated animals began to lose weight only at the advanced stage of the disease.

There was no parallelism between weight loss and prolongation of life. In Fig. 2 (Table III, experiment 9) it is true that the controls gained most weight and died first, but the mapharsen- and arsenite-treated mice had similar weight curves, although the arsenite-treated mice survived twice as long as the mapharsen-treated animals and neither group of mice had a significant weight loss at the time of death of the controls.

*Differences in susceptibility of strains of leukemia to therapeutic agents.*—This is well shown in Fig. 1. In myeloid leukemia 106 both benzene and arsenite significantly prolonged the lives of the leukemic mice, and underfeeding was almost as effective. In chloroleukemia 1394 benzene often more than tripled the time of survival of the leukemic animals, while underfeeding and x-rays had only a moderate effect, and arsenite none at all. On myeloid leukemia 1712 no agent tested other than x-rays was effective, and these prolonged life only slightly.

The therapeutic effects were, on the whole, more striking with myeloid than with lymphoid strains. However, the lymphoid strains were not as adequately tested as the myeloid. Furthermore, the myeloid strains were, in general, more chronic than the lymphoid strains, and the results do not warrant any generalization as to the relative susceptibility of the lymphoid and myeloid leukemias to the agents tested. It would also seem desirable to study monocytic leukemias

and aleukemic histiocytic neoplasms (so called reticulum cell sarcomas).

In our experiments the more acute strains did not respond to therapy less favorably than the more chronic strains. The duration of illness under the conditions of the experiments varied with different strains from approximately 10 days to approximately 7 weeks. While a course of 10 days is fairly rapid, the gross changes of leukemia were evident at autopsy, and these leukemias of short duration may not be comparable to acute leukemia in man.

*Time of administration of chemical.*—Since the disease in animals is seldom discovered before it is far advanced, in most experiments the administration of the chemical was begun shortly after inoculation. It seemed highly desirable, however, to apply chemicals that were found useful in such preliminary experiments at the more advanced stages of the disease. This was done in a few experiments. The results with chloroleukemia 1394, shown in experiment 23 (Table V), are particularly illuminating. They indicate that even when the disease is so far advanced that the controls begin to die, benzene may still conspicuously prolong the lives of some animals. The response of the animals to late treatment is usually one of two extremes: (a) death shortly after administration, (b) long survival. This may be explained by assuming that if the disease was too far advanced the damage was already too severe to permit survival, while in the remaining animals, whose lives were definitely lengthened, a sufficient number of leukemic cells were destroyed or inhibited in multiplication, and life was conspicuously prolonged. The observations of Kalapos (16) are noteworthy in this connection. This investigator believes that benzene acts more promptly than x-ray. Should this be true, benzene may be more effective at the advanced stage of the disease than x-rays. Further observations are needed on the effectiveness of the delayed administration of benzene and allied chemicals in the prolongation of life in leukemic mice.

It is desirable to investigate the fundamental problem of the mode of action of benzene and arsenite on leukemic cells. A closer consideration of the pharmacological action of the useful drugs is essential, for broadening of knowledge may lead to the discovery of better compounds or to more efficient application of those already known.

It is remarkable that benzene and potassium arsenite were found as, or more, effective than any other arsenical or benzene derivative hitherto tried. But *a priori* it seems likely that better compounds exist. On one hand, it seems advisable to extend these investigations in a systematic manner to other ring compounds of different structure, to other benzene derivatives, to different arsenicals, and to compounds related to arsenic such as antimony and phosphorus. Radioactive arsenicals could be tried for comparison. On the other hand, exploration should also be made on a "hit or miss" basis with entirely different chemicals.

If such results as these are to be applied to therapy in man, it will be necessary to devise tests to indicate which of the chemicals or procedures is likely to be more beneficial in a given case. Tissue cultures may serve for this purpose.

## SUMMARY

The effect of underfeeding, of x-rays, and of the administration of potassium arsenite, four organic arsenicals (neoarsphenamine, mapharsen, tryparsamide, and soamine), of benzene and benzene derivatives (toluene, three xylenes, mesitylene, pseudocumene, ethylbenzene, and cymene), and stilbestrol, sulfadiazine, and sulfaguanidine has been studied on transmitted myeloid and lymphoid leukemias in mice.

Each strain of myeloid leukemia responded differently to the therapeutic agents, but the prolongation of life of the leukemic mice caused by the administration of a given chemical or other agent was fairly constant for each strain. Benzene, potassium arsenite, and, to a lesser extent, underfeeding retarded the development of one strain of myeloid leukemia (No. 106). All three were ineffective against another strain (No. 1712), while only benzene was highly effective against a myeloid chloroleukemia (strain 1394). X-ray had some effect on the last two strains. The best results were obtained with benzene, which occasionally doubled or tripled the period of survival of the animals. Some beneficial effect was manifest even when benzene was administered to mice with myeloid chloroleukemia (strain 1394) at an advanced stage of the disease.

Benzene, underfeeding, and x-ray all prolonged the lives of mice with certain strains of lymphoid leukemia. The response was not related to the chronicity of the disease.

No organic arsenical or benzene derivative was as effective as arsenite or benzene.

Underfeeding alone retarded the development of most strains of leukemia, but many of the chemicals seemed to exert their beneficial effect without causing loss of weight.

While sulfadiazine and sulfaguanidine did not significantly prolong the life of the leukemic mice, they did not harm the animals. These compounds may be of value in the treatment of infections complicating leukemias.

The authors gratefully acknowledge the able assistance of Miss Sylvia Schutz, M. A., and Miss Mary C. Boon, M. A.

REFERENCES


1. Barnes, W. A., and Sisman, I. E. Myeloid Leukemia and Nonmalignant Extramedullary Myelopoiesis in Mice. Am. J. Cancer, **37**:1-35. 1939.
2. Boyland, E. Experiments on the Chemotherapy of Cancer. 1. Effect of Certain Antibacterial Substances and Related Compounds. Biochem. J., **32**:1207-1213. 1938.
3. Boyland, E. Experiments on the Chemotherapy of Cancer. 5. The Effect of Muscle Extract and Aliphatic Bases. Biochem. J., **35**:1283-1288. 1941.
4. Boyland, E., and Mawson, E. H. Experiments on the Chemotherapy of Cancer. 2. Effect of Aldehydes and Glucosides. Biochem. J., **32**:1982-1987. 1938.
5. Breedis, C. Action of Extreme Cold on Leukemic Cells of Mice. J. Exper. Med., **76**:221-240. 1942.
6. Breedis, C., and Furth, J. The Feasibility of Preserving Neoplastic Cells in the Frozen State. Science, **88**: 531-532. 1938.
7. Forkner, C. E. Leukemia and Allied Disorders. New York: Macmillan Company. 1938.
8. Furth, J. Studies on the Effect of Roentgen Rays on Lymphomatosis of Mice. Am. J. Roentgenol., **32**:377-383. 1934.
9. Furth, J., and Kabakjian, D. H. Studies on the Effect of Continuous Exposure of Mice to Gamma Rays of Radium. Am. J. Roentgenol., **32**:227-234. 1934.
10. Furth, J., and Kahn, M. C. The Transmission of Leukemia of Mice with a Single Cell. Am. J. Cancer, **31**: 276-282. 1937.
11. Furth, J., Rathbone, R. R., and Seibold, H. R. Relation of X-Rays to Lymphomatosis. Proc. Soc. Exper. Biol. & Med., **30**:754-756. 1933.
12. Furth, J., Seibold, H. R., and Rathbone, R. R. Experimental Studies on Lymphomatosis of Mice. Am. J. Cancer, **19**:521-592. 1933.
13. Furth, J., Tuggle, A., and Breedis, C. Quantitative Studies on the Effect of X-Rays on Neoplastic Cells. Proc. Soc. Exper. Biol. & Med., **38**:490-492. 1938.
14. Hall, J. W., and Knocke, F. J. Transmission of Chloroleukemia of Mice. Am. J. Path., **14**:217-225. 1938.
15. Hunter, F. T. Chronic Exposure to Benzene (Benzol). II. The Clinical Effects. J. Indust. Hyg. & Toxicol., **21**: 331-354. 1939.
16. Kalapos, I. Die Wirkung des Benzols bei der Leukämie. Klin. Wchnschr., **14**:864-867. 1935.
17. Korányi, A. Der Beeinflussung der Leukämie durch Benzol. Berl. klin. Wchnschr., **49**:1357-1358. 1912.
18. Lignac, G. O. E. Blastomartige Erkrankung des weissen Maus durch chronische Benzolvergiftung und ihre Beziehung zur Leukämie. Krankheitsforschung, **6**:97-130. 1928.
19. Lignac, G. O. E. Die Benzolleukämie bei Menschen und weissen Mäusen. Klin. Wchnschr., **12**:109-110. 1933.
20. Lits, F. J., Kirschbaum, A., and Strong, L. C. Action of Colchicine on a Malignant Lymphoid Neoplasm in Mice of an Inbred Strain. Proc. Soc. Exper. Biol. & Med., **38**: 555-557. 1938.
21. Mallory, T. B., Gall, E. A., and Brickley, W. J. Chronic Exposure to Benzene (Benzol). III. The Pathologic Results. J. Indust. Hyg. & Toxicol., **21**:355-393. 1939.
22. Maver, M. E., and Voegtlin, C. The Chemistry of Cell Division. VI. The Inhibition of Tumor and Liver Nuclease by Arsenicals. Am. J. Cancer, **29**:333-340. 1937.
23. McCay, C. M., Ellis, G. H., Barnes, L. L., Smith, C. A. M., and Sperling, G. Chemical and Pathological Changes in Aging and after Retarded Growth. J. Nutrition, **18**:15-25. 1939.
24. Morris, H. P., and Thompson, J. W. Convenient Inexpensive Device for Quantitative Hand Feeding of Mice. J. Nat. Cancer Inst., **1**:395-396. 1940.
25. Naegeli, O. Zur Behandlung der Anämien und Leukämien. Wien. med. Wchnschr., **80**:457-460. 1930.
26. Saxton, J. A. An Experimental Study of Nutrition and Age as Factors in the Pathogenesis of Common Diseases of the Rat. Abstract. New York State J. Med., **41**:1095-1096. 1941.
27. Saxton, J. A., and Furth, J. The Effect of Underfeeding on the Spontaneous Leukemias of Mice. Paper in preparation.
28. Sollman, T. Manual of Pharmacology. Fourth edition. Philadelphia and London: W. B. Saunders Company. 1932, p. 975.
29. Strong, L. C. The Synergistic Effect of Heptyl Aldehyde and Methyl Salicylate on Spontaneous Tumors of the Mammary Gland in Mice. Yale J. Biol. & Med., **11**: 207-218. 1938-39.
30. Sugiura, K., and Benedict, S. R. The Influence of Insufficient Diets upon Tumor Recurrence and Growth in Rats and Mice. J. Cancer Research, **10**:309-318. 1926.
31. Tannenbaum, A. The Initiation and Growth of Tumors. Introduction. I. Effects of Underfeeding. Am. J. Cancer, **38**:335-350. 1940.
32. Tannenbaum, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction *per se*. Cancer Research, **2**:460-467. 1942.
33. Voegtlin, C., Dyer, H. A., and Leonard, C. S. On the Mechanism of the Action of Arsenic upon Protoplasm. Pub. Health Rep., **38**:1882-1912. 1923.
34. Weil, P. E., and Perlés, S. Un cas de leucémie aiguë chez un benzolique. Sang, **14**:270-272. 1940.

# Chemical Studies on the Mode of Action of Methylcholanthrene on Mouse Epidermis*

Christopher Carruthers, Ph.D., and V. Suntzeff, M.D.

*(From the Department of Research, The Barnard Free Skin and Cancer Hospital, and the Department of Anatomy, Washington University School of Medicine, St. Louis, Mo.)*

(Received for publication June 7, 1943)

Previous studies (2, 6) have shown that methylcholanthrene produced a notable decrease in the iron and calcium content of mouse epidermis while the sodium and ascorbic acid content was not significantly altered. For a more complete understanding of the events occurring in the epidermis under the influence of this carcinogen, the effect of the latter on epidermal magnesium and potassium was investigated.

## The Mineral Content of Mouse Epidermis

The mineral content of mouse epidermis is detailed in Table I. The potassium content is more than twice that of the sodium, and these metals account for 88 per cent of the total cations. Calcium and magnesium occur in the ratio of about 2 to 1, and make up 11 per cent of the metals. On the other hand iron is low, and preliminary experiments with large amounts (400 to 800 mgm. of epidermis from 15 to 20 mice) indicate a copper content of 0.2 to 0.4 microgram per 100 mgm. Since the epidermis is avascular inorganic analysis is facilitated owing to the noninterference of blood. This fact is well illustrated in Table I by the excellent agreement in the normal content of each of the metals in different samples.

The methods of separating the epidermis from the dermis were devised by Baumberger, Suntzeff, and Cowdry (1), and the adaptation of microchemical methods to the small amounts of tissue thus obtained should be of great value in skin physiology and in dermatology. Microchemical methods are essential because only limited amounts of epidermis can be obtained unless many mice are sacrificed or large pieces of human or other skin are removed. For example, only 150 to 200 mgm. of epidermis can be secured from the entire backs of 10 to 12 mice at the age of 3 to 4 months. After incineration there is but 1 to 2 mgm. of ash left for inorganic analysis.

* This investigation was aided by grants from The International Cancer Research Foundation and the National Cancer Institute.

## Experimental Procedure

New Buffalo mice of both sexes and 3 to 4 months old were used, as there was no apparent sex difference. The methods of shaving, applying the carcinogen, and of separating the epidermis from the dermis have been described (2). In this investigation, as in those already completed, nucleoprotein phosphorus was used as a basis of reference for the amount of tissue in-

Table I: The Mineral Composition of Mouse Epidermis

| Metal | Number of mice represented | Mgm. metal per 100 mgm. epidermis | Total cations, per cent |
|---|---|---|---|
| Potassium | 10 | 0.3480 | |
| | 10 | 0.3475 | |
| | 8 | 0.3400 | |
| | 10 | 0.3507 | |
| | 38 (total) | | |
| | Average | 0.3465 | 59.4 |
| Sodium | 8 | 0.1707 | |
| | 7 | 0.1697 | |
| | 8 | 0.1490 | |
| | 8 | 0.1829 | |
| | 8 | 0.1677 | |
| | 39 (total) | | |
| | Average | 0.1680 | 28.7 |
| Calcium | 7 | 0.0358 | |
| | 8 | 0.0420 | |
| | 11 | 0.0505 | |
| | 11 | 0.0400 | |
| | 10 | 0.0405 | |
| | 12 | 0.0520 | |
| | 59 (total) | | |
| | Average | 0.0435 | 7.5 |
| Magnesium | 12 | 0.0195 | |
| | 12 | 0.0176 | |
| | 11 | 0.0210 | |
| | 10 | 0.0164 | |
| | 45 (total) | | |
| | Average | 0.0186 | 3.3 |
| Iron | 108 (total) | | |
| | Average | 0.0064 | 1.0 |

volved. The necessity of an adequate base in these studies has been given (2).

The magnesium content of the epidermis was determined by a micro-polarographic method (3) devised in this laboratory. The potassium content was determined as follows: The sample was ashed at 450° C. in a silica crucible in a muffle furnace until all the organic matter had been destroyed. The ash remaining was dissolved with 1 cc. of 0.1 *N* hydrochloric acid and the crucible contents were rinsed with several 2 cc. portions of distilled water into a pyrex test tube. The solution in the test tube was evaporated to dryness in a beaker of boiling water, and after cooling to room temperature, sufficient platinic chloride (5 per cent solution in 0.1 *N* hydrochloric acid) was added to combine with all the potassium and sodium present. The resulting mixed solution of platinates was evaporated to dryness as before and the residue remaining was extracted with 2 to 4 cc. of 80 per cent alcohol at room temperature. The test tube was stoppered and allowed to stand overnight to insure complete precipitation of the potassium chloroplatinate. Then the latter compound was separated from the mother liquor by aspiration with a pyrex filter stick (10 mm. disk, medium porosity) covered with a layer of asbestos. The precipitate of potassium chloroplatinate was washed with two 2 cc. portions of freshly prepared alcohol wash solution. The wash solution of 95 per cent alcohol was saturated with potassium chloroplatinate and filtered just before use through a sintered glass filter covered with a layer of asbestos. The rest of the procedure was that of Consolazio and Talbott (4) except that 0.005 *N* sodium thiosulfate was used to titrate (from a microburette) the potassium iodoplatinate.

## RESULTS

### Magnesium

A comparison of the analysis for magnesium of normal controls, benzene-treated controls, and methylcholanthrene-treated mice is shown in Table II. The results are expressed in terms of milligrams of magnesium (Mg) per 100 mgm. of tissue, of milligrams of nucleoprotein phosphorus (denoted by N.P.P.) per 100 mgm. of tissue, and by the significant ratio Mg/N.P.P. × 10 (hereinafter denoted by Mg/N.P.P.). The epidermis of normal mice contained an average of 0.0186 mgm. Mg per 100 mgm. and had an Mg/N.P.P. ratio of 1.44. The benzene-treated epidermis had an average of 0.0189 mgm. Mg per 100 mgm. and an Mg/N.P.P. ratio of 1.53. The effect of benzene alone was therefore not significant. After 1 and 2 applications of methylcholanthrene the magnesium content and the ratios, Mg/N.P.P., were slightly greater than those of the normal. However,

Table II: Magnesium/Nucleoprotein Phosphorus Ratio of Mouse Epidermis

| Number of mice | Number of paintings | Time after first treatment to killing of mice, days | Mg per 100 mgm. tissue, mgm. | N.P.P. per 100 mgm. tissue, mgm. | Mg/N.P.P × 10 |
|---|---|---|---|---|---|
| | | | NORMAL, UNTREATED MICE | | |
| 12 | | | 0.0195 | 0.127 | 1.53 |
| 12 | | | 0.0176 | 0.139 | 1.26 |
| 11 | | | 0.0210 | 0.121 | 1.73 |
| 10 | | | 0.0164 | 0.133 | 1.23 |
| 45 | (total) | | | | |
| | Average | | 0.0186 | 0.130 | 1.44 |
| | | | BENZENE-TREATED MICE | | |
| 12 | 3 | 10 | 0.0193 | 0.116 | 1.66 |
| 11 | 3 | 10 | 0.0178 | 0.139 | 1.28 |
| 11 | 3 | 10 | 0.0184 | 0.127 | 1.45 |
| 12 | 3 | 10 | 0.0204 | 0.113 | 1.80 |
| 46 | (total) | | | | |
| | Average | | 0.0189 | 0.124 | 1.55 |
| | | | METHYLCHOLANTHRENE-TREATED MICE | | |
| 8 | 1 | 10 | 0.0209 | 0.139 | 1.50 |
| 7 | 1 | 10 | 0.0215 | 0.134 | 1.60 |
| 8 | 1 | 10 | 0.0202 | 0.119 | 1.70 |
| 23 | (total) | | | | |
| | Average | | 0.0208 | 0.131 | 1.60 |
| 6 | 2 | 10 | 0.0228 | 0.140 | 1.63 |
| 8 | 3 | 10 | 0.0271 | 0.134 | 2.02 |
| 8 | 3 | 10 | 0.0311 | 0.151 | 2.06 |
| 6 | 3 | 10 | 0.0220 | 0.148 | 1.48 |
| 8 | 4 | 10 | 0.0207 | 0.121 | 1.71 |
| 30 | (total) | | | | |
| | Average | | 0.0252 | 0.138 | 1.82 |
| 6 | 4 | 15 | 0.0229 | 0.149 | 1.53 |
| 6 | 4 | 15 | 0.0207 | 0.142 | 1.52 |
| 12 | (total) | | | | |
| | Average | | 0.0218 | 0.145 | 1.53 |
| 7 | 6 | 20 | 0.0235 | 0.142 | 1.65 |
| 5 | 6 | 20 | 0.0266 | 0.149 | 1.80 |
| 9 | 6 | 20 | 0.0208 | 0.138 | 1.50 |
| 21 | (total) | | | | |
| | Average | | 0.0236 | 0.143 | 1.65 |
| 8 | 12 | 30 | 0.0219 | 0.124 | 1.76 |
| 8 | 12 | 30 | 0.0193 | 0.116 | 1.66 |
| 5 | 12 | 30 | 0.0216 | 0.119 | 1.81 |
| 21 | (total) | | | | |
| | Average | | 0.0209 | 0.119 | 1.74 |
| 7 | 24 | 60 | 0.0196 | 0.112 | 1.75 |
| 4 | 24 | 60 | 0.0214 | 0.120 | 1.78 |
| 4 | 24 | 60 | 0.0195 | 0.120 | 1.62 |
| 4 | 24 | 60 | 0.0248 | 0.117 | 2.12 |
| 4 | 24 | 60 | 0.0189 | 0.117 | 1.61 |
| 23 | (total) | | | | |
| | Average | | 0.0208 | 0.117 | 1.77 |

after 3 treatments the magnesium had increased to 0.0252 mgm. (average) per 100 mgm. and the ratio Mg/N.P.P. to 1.82. Likewise the epidermis treated 6 times with the carcinogen showed an elevated Mg content and Mg/N.P.P. ratio.

In order to determine whether the increase in magnesium was significant, samples were removed at 15 days after 4 applications of the carcinogen. However, both the Mg content and Mg/N.P.P. ratios were only slightly above normal.

After 12 and 24 treatments of mice that were killed at 30 and 60 days respectively, there was excellent agreement in the magnesium content and in the Mg/N.P.P. ratios, the latter being about 20 per cent higher than those of the normal controls. The importance of the increase in the Mg/N.P.P. ratios of most of the methylcholanthrene-treated mice may be of questionable significance since mice treated 4 times had a nearly normal ratio. Moreover, the epidermises having the greatest rise (after 3 and 6 applications and killed at 10 and 20 days respectively) in magnesium and in Mg/N.P.P. ratios showed considerable variation.

## Potassium

The results for potassium are given in Table III, and are expressed in the same manner as those for magnesium except that the ratio K/N.P.P. was not multiplied by 10. Normal epidermis contained an average of 0.3465 mgm. potassium per 100 mgm. and had a K/N.P.P. ratio of 2.68, and the benzene-treated had an average of 0.3511 mgm. potassium per 100 mgm. and a K/N.P.P. ratio of 2.64. It was evident that benzene alone had no effect on epidermal potassium. Mice killed at 10 days after 1 and 3 applications of methylcholanthrene did not show any appreciable change in the K content or in the K/N.P.P. ratios. Likewise the epidermises removed at 20, 30, and 60 days after 6, 12, and 24 treatments respectively had nearly normal K range and K/N.P.P. ratios. Therefore epidermal potassium was not altered under the influence of the carcinogen.

## DISCUSSION

The effect of methylcholanthrene upon epidermal iron, sodium, calcium, magnesium, potassium, and ascorbic acid is shown graphically in Fig. 1. The time in days is plotted against the percentage of change in the metal/nucleoprotein phosphorus ratios. The number of applications of the carcinogen corresponding to various times in days is expressed at the top of the graph. As early as 10 days after 1 application of methylcholanthrene the Fe and Ca/N.P.P. ratios had dropped to about 50 per cent of the normal. There was a further reduction in the Fe/N.P.P. ratio,

Table III: Potassium/Nucleoprotein Phosphorus Ratio of Mouse Epidermis

| Number of mice | Number of paintings | Time after first treatment to killing of mice, days | K per 100 mgm. tissue, mgm. | N.P.P. per 100 mgm. tissue, mgm. | K/N.P.P. |
|---|---|---|---|---|---|
| | | | NORMAL, UNTREATED MICE | | |
| 10 | | | 0.3480 | 0.128 | 2.72 |
| 10 | | | 0.3475 | 0.124 | 2.80 |
| 8 | | | 0.3400 | 0.129 | 2.64 |
| 10 | | | 0.3507 | 0.136 | 2.58 |
| 38 | (total) | | | | |
| | Average | | 0.3465 | 0.129 | 2.68 |
| | | | BENZENE-TREATED MICE | | |
| 10 | 3 | 10 | 0.3547 | 0.125 | 2.84 |
| 9 | 3 | 10 | 0.3503 | 0.121 | 2.89 |
| 10 | 3 | 10 | 0.3313 | 0.153 | 2.16 |
| 9 | 3 | 10 | 0.3681 | 0.136 | 2.70 |
| 38 | (total) | | | | |
| | Average | | 0.3511 | 0.134 | 2.65 |
| | | | METHYLCHOLANTHRENE-TREATED MICE | | |
| 6 | 1 | 10 | 0.3242 | 0.109 | 2.97 |
| 6 | 1 | 10 | 0.3220 | 0.136 | 2.37 |
| 6 | 1 | 10 | 0.3642 | 0.156 | 2.34 |
| 18 | (total) | | | | |
| | Average | | 0.3368 | 0.134 | 2.56 |
| 5 | 3 | 10 | 0.3615 | 0.126 | 2.87 |
| 6 | 3 | 10 | 0.3430 | 0.106 | 3.23 |
| 6 | 3 | 10 | 0.3609 | 0.149 | 2.42 |
| 17 | (total) | | | | |
| | Average | | 0.3551 | 0.127 | 2.84 |
| 6 | 6 | 20 | 0.3870 | 0.132 | 2.93 |
| 5 | 6 | 20 | 0.3180 | 0.131 | 2.43 |
| 6 | 6 | 20 | 0.3815 | 0.148 | 2.58 |
| 6 | 6 | 16 | 0.3553 | 0.135 | 2.63 |
| 23 | (total) | | | | |
| | Average | | 0.3604 | 0.136 | 2.64 |
| 8 | 12 | 30 | 0.3456 | 0.140 | 2.47 |
| 3 | 12 | 30 | 0.3504 | 0.119 | 2.94 |
| 4 | 12 | 30 | 0.3526 | 0.119 | 2.96 |
| 3 | 12 | 30 | 0.3310 | 0.123 | 2.69 |
| 4 | 12 | 30 | 0.3280 | 0.123 | 2.66 |
| 22 | (total) | | | | |
| | Average | | 0.3415 | 0.125 | 2.73 |
| 4 | 24 | 60 | 0.3329 | 0.125 | 2.66 |
| 4 | 24 | 60 | 0.3401 | 0.125 | 2.72 |
| 4 | 24 | 60 | 0.3284 | 0.130 | 2.52 |
| 6 | 24 | 50 | 0.3546 | 0.131 | 2.70 |
| 18 | (total) | | | | |
| | Average | | 0.3390 | 0.128 | 2.65 |

while the Ca/N.P.P. ratio diminution remained fairly constant. The decrease in iron may be associated with a lowering of the cytochrome *c* content, since the latter was found to be less in neoplastic growths (5) than in the normal tissues from which the malignant growths arose. The chemical changes, if any, associated with the lowering of calcium must await further investigation.

ments the ratio was essentially normal. Although the late hyperplasias (30 and 60 days) showed about a 20 per cent augmentation in the ratios, it was difficult to evaluate the rise owing to the variation in the Mg content of different samples. The K/N.P.P. ratio was notably constant throughout the period of carcinogenesis.

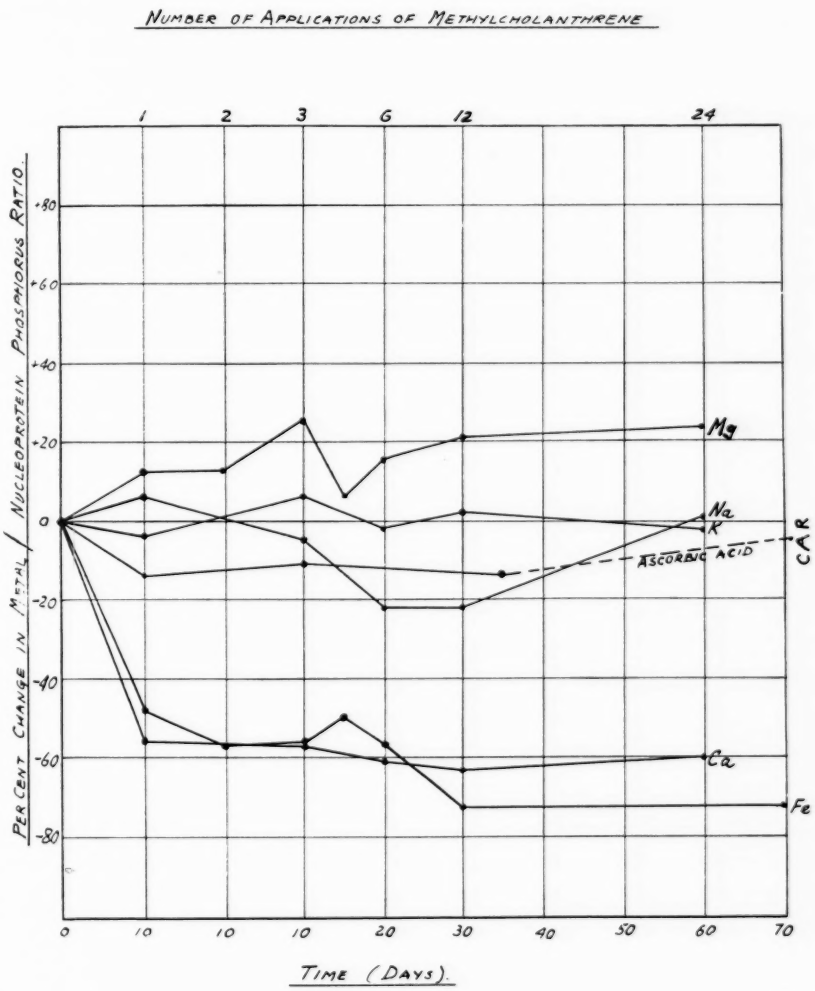



FIG. 1.—The influence of methylcholanthrene on epidermal iron (Fe), sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), and ascorbic acid.

On the other hand the ascorbic acid/N.P.P. ratio was not appreciably affected. This constituent was also determined in epidermal carcinomas (denoted on the graph by CAR) and the ratio was found to be nearly the same as that of normal epidermis. At 20 and 30 days after 6 and 12 treatments respectively the decrease in the Na/N.P.P. ratio was about 20 per cent, but at 60 days the ratio was normal. It was therefore concluded that epidermal sodium was not significantly altered. The Mg/N.P.P. ratio was increased by nearly 25 per cent at 10 and 20 days after 3 and 6 applications respectively, but at 15 days after 4 treat-

$\frac{\text{K/N.P.P.}}{\text{Na/N.P.P.}}$ and $\frac{\text{Ca/N.P.P.}}{\text{Mg/N.P.P.}}$ ratios of normal and methylcholanthrene-treated epidermis are shown in Table IV. These data further demonstrate that potassium and sodium were not significantly changed in the process of carcinogenesis although the ratio was increased somewhat in the epidermis treated 6 and 12 times. On the other hand, the Ca/Mg ratio decreased by more than 60 per cent after the application of methylcholanthrene, the diminution remaining remarkably constant from 10 to 60 days. It is obvious, therefore, that the alkaline earths, especially

calcium, play an important role in the transformation of normal to "precancerous" tissue.

The data expressed graphically in Fig. 1 also demonstrate that the response of mouse epidermis to methylcholanthrene is rapid and chemically complex even in 10 day samples following a single treatment with the carcinogen. Wicks and Suntzeff (7) have also shown that the total lipid/protein nitrogen ratio of mouse epidermis was much lowered as early as 5 days after one application of methylcholanthrene. Therefore it is apparent that the process of epidermal carcinogenesis in mice is initiated early, and that there

TABLE IV: POTASSIUM-SODIUM/NUCLEOPROTEIN PHOSPHORUS RATIO AND CALCIUM-MAGNESIUM/NUCLEOPROTEIN PHOSPHORUS RATIO OF MOUSE EPIDERMIS

| Treatment | Number of paintings | Time after first treatment to killing of mice, days | K/N.P.P. / Na/N.P.P. | Ca/N.P.P. / Mg/N.P.P. |
|---|---|---|---|---|
| Normal | | 10 | 2.29 | 2.41 |
| Benzene-treated | 3 | 10 | 2.18 | 2.19 |
| Methylcholanthrene-treated | 1 | 10 | 2.05 | 0.93 |
| " " | 3 | 10 | 2.55 | 0.85 |
| " " | 6 | 20 | 2.90 | 0.85 |
| " " | 12 | 30 | 3.04 | 0.72 |
| " " | 24 | 60 | 2.24 | 0.82 |

are at least several chemical changes occurring simultaneously.

These investigations also reveal from a chemical standpoint the existence of "precancerous" tissue. This is apparent from Fig. 1, which shows that the methylcholanthrene-treated cells have lost 50 per cent of their calcium and iron, and about the same amount of total lipid (7), and also may be richer in magnesium. Nevertheless these cells are capable of continued existence and proliferation to form extensive hyperplasias and carcinomas. It is not yet known whether the mineral changes are maintained in epidermal squamous cell carcinomas.

SUMMARY

The effect of methylcholanthrene upon epidermal iron, calcium, sodium, magnesium, potassium, and ascorbic acid is discussed. One application of the carcinogen reduced within 10 days the iron and calcium content to approximately 50 per cent of the normal. On the other hand sodium, potassium, and ascorbic acid were not appreciably altered, while the increase in magnesium may be important. The significance of the chemical changes is briefly outlined. Nucleoprotein phosphorus was used as a basis of reference for the amount of tissue involved.

The existence of "precancerous" tissue from a chemical standpoint is postulated.

The mineral analysis of mouse epidermis is also given, and the use of the technics employed in these studies for other biological problems is briefly mentioned.

REFERENCES

1. BAUMBERGER, J. P., SUNTZEFF, V., and COWDRY, E. V. Methods for the Separation of Epidermis from Dermis and Some Physiologic and Chemical Properties of Isolated Epidermis. J. Nat. Cancer Inst., **2**:413-423. 1942.
2. CARRUTHERS, C., and SUNTZEFF, V. Influence of Limited Application of Methylcholanthrene upon Epidermal Iron and Ascorbic Acid. J. Nat. Cancer Inst., **3**:217-220. 1942.
3. CARRUTHERS, C. Microdetermination of Magnesium with the Polarograph. Indust. & Engin. Chem. (Anal. Ed.), **15**: 412-414. 1943.
4. CONSOLAZIO, W. V., and TALBOTT, J. H. Modification of the Method of Shohl and Bennett for the Determination of Potassium in Serum and Urine. J. Biol. Chem., **126**: 55-61. 1938.
5. DUBOIS, K. P., and POTTER, V. R. Biocatalysts in Cancer Tissue. I. Cytochrome *c*. Cancer Research, **2**:290-293. 1942.
6. SUNTZEFF, V., and CARRUTHERS, C. The Effect of Methylcholanthrene on Epidermal Sodium and Calcium. Cancer Research, **3**:431-433. 1943.
7. WICKS, L. F., and SUNTZEFF, V. Reduction of Total Lipid-Protein Nitrogen Ratio of Mouse Epidermis by a Single Application of Methylcholanthrene. J. Nat. Cancer Inst., **3**:221-226. 1942.

# Further Studies on the Tumor-Promoting Action of Fat*†

P. S. Lavik, Ph.D., and C. A. Baumann, Ph.D.

*(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis.)*

(Received for publication June 21, 1943)

There is abundant evidence that diets high in fat tend to increase the rate of formation of certain types of induced tumors (1, 2, 4, 18). In the presence of borderline amounts of carcinogen, dietary fat may also increase the total number of tumors produced (6). Not all types of tumors, however, are sensitive to dietary fat (2, 9, 18), and at least a part of the effect appears to be due to local fat in direct contact with the area in which the tumor develops (4, 11, 13, 20). The present report deals with further studies on the extent and limitation of the "fat effect," and includes attempts to explain the mechanism of action involved.

## METHOD

The experimental procedure was essentially that reported previously (6). Tumors were produced on the backs of albino mice by the application twice weekly of a 0.2 per cent solution of methylcholanthrene in dioxane. The experiments were performed in a number of series in each of which the animals were divided into groups of 25, comparable in age and weight. All groups within a series were treated with the same amount of hydrocarbon, but each in addition received some special dietary treatment. Except in certain specific experiments food and water were given *ad libitum*, and all mice were examined twice monthly for tumors.

A basal ration used in compounding many of the diets had the following composition:

| | |
|---|---|
| Corn meal | 445 |
| Skim milk powder | 222 |
| Linseed oil meal | 167 |
| Soybean oil meal | 111 |
| Alfalfa leaf meal | 33 |
| NaCl (fine, iodized) | 11 |
| $Ca_3(PO_4)_2$ | 11 |

A diet relatively low in fat was used as the control ration in most of the experimental series; it consisted of 93 parts of the basal ration, 5 parts of brewers' yeast, and 2 parts of cod liver oil. Diets high in fat were prepared from the same ingredients with fat replacing an equivalent amount of the basal ration. When still other ingredients were included in the diet, they were likewise substituted at the expense of the basal ration. Semisynthetic diets were prepared as indicated in the separate sections dealing with the experiments themselves.

## EXPERIMENTAL

*Synthetic triglycerides.*—It was observed previously that ethyl laurate stimulated the production of tumors to nearly the same extent as the natural fats themselves, while glycerol and the unsaponifiable matter from vegetable oil increased tumor formation only slightly (6). In the present studies, fatty acids from hydrogenated vegetable oil (primex) were prepared free from unsaponifiable matter, resynthesized into triglycerides (7), and fed at a level of 10 per cent to mice receiving the usual carcinogenic treatment. The percentage of tumors that developed in these animals was found to be the same as on diets containing 10 per cent of natural fat—37 to 43 per cent at 6 months, as compared to 18 per cent on the low fat control diet (Table I). It appeared, therefore, that neither the sterols nor the other substances in unsaponifiable matter were necessary for demonstrating the tumor-promoting effect of fat.

TABLE I: THE EFFECT OF SYNTHETIC TRIGLYCERIDES ON THE FORMATION OF INDUCED SKIN TUMORS IN MICE

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

| Diet | Effective total | Tumor formation 2 mos., per cent | 4 mos., per cent | 6 mos., per cent |
|---|---|---|---|---|
| Low fat control | 22 | 0 | 4 | 18 |
| 10% lard (m.p. $>$ 37° C.) | 23 | 0 | 30 | 43 |
| 10% synthetic triglycerides * | 22 | 0 | 27 | 41 |
| 10% lard (m.p. $<$ 37° C.) | 24 | 0 | 25 | 37 |

* Synthetic triglycerides of fatty acids from hydrogenated vegetable oil (primex).

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† This investigation was supported in part by the Wisconsin Alumni Research Foundation and by the Jonathan Bowman Cancer Fund. Most of the experiments described in this paper were included in a thesis "Dietary Factors Affecting Tumor Formation" submitted by P. S. Lavik in partial fulfillment of the degree of Doctor of Philosophy, University of Wisconsin, January, 1943.

*Effect of heating the fat.*—The heated vegetable oil, primex, has been reported to be more effective in increasing tumor formation than the unheated fat. Subsequent experiments, however, indicated that the original results were not representative for heated fats in general. Lard, coconut oil, and corn oil were heated at 300° C. for 1 hour as previously described (6), incorporated into the basal ration as 10 per cent of the diet, and fed to mice receiving the usual carcinogenic treatment. The incidence of tumors in these groups was then compared with that in 3 others fed 10 per cent of the respective unheated fats. In addition, another group of mice was fed a diet containing 10 per cent of coconut oil plus 1 per cent of cholesterol that had been heated to 300° C. for 1 hour.

Little difference was found between the stimulatory activity of the heated and the unheated fats, and at the end of 5 months the tumor incidence in each of the groups varied from 61 to 78 per cent (Table II). Furthermore, when 1 per cent of heated cholesterol was added to the diet containing coconut oil, the tumor incidence was no greater than when coconut oil alone was fed. At a level of 7 per cent, primex and lard heated to 300° C. for 4 hours were less effective in increasing tumor formation than the corresponding unheated fats (Table II, groups 9 to 13). However, this apparent reduction in the ability of heated fats to stimulate tumor formation was not interpreted as a genuine effect, but rather as the result of a reduced consumption of the diet by the mice (see below), presumably because of disagreeable substances present in the heated fats.

TABLE II: THE EFFECT OF HEATED FAT ON TUMOR FORMATION

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

| Group | Diet | Effective total | Tumor formation: 2 mos., per cent | 4 mos., per cent | 5 mos., per cent |
|---|---|---|---|---|---|
| 1 | Low fat control | 21 | 0 | 28 | 33 |
| 2 | 10% corn oil | 21 | 19 | 71 | 76 |
| 3 | 10% heated corn oil | 24 | 16 | 71 | 71 |
| 4 | 10% coconut oil | 24 | 4 | 66 | 66 |
| 5 | 10% heated coconut oil | 23 | 4 | 70 | 78 |
| 6 | 10% coconut oil + 1% heated cholesterol | 22 | 9 | 63 | 68 |
| 7 | 10% lard | 18 | 11 | 61 | 61 |
| 8 | 10% heated lard | 16 | 26 | 69 | 69 |
| 9 | Low fat control | 22 | 0 | 23 | 27 * |
| 10 | 7% primex † | 17 | 0 | 59 | 59 * |
| 11 | 7% heated primex | 22 | 0 | 27 | 32 * |
| 12 | 7% lard | 26 | 8 | 38 | 42 * |
| 13 | 7% heated lard | 25 | 0 | 20 | 24 * |

* Tumor incidence at 6 months.

† Hydrogenated vegetable oil.

*Tumors relatively insensitive to dietary fat.*—Tannenbaum has recently reported that diets high in fat stimulate the formation of induced skin tumors and of spontaneous breast tumors in mice, but not of primary lung tumors nor of induced subcutaneous sarcomas (18). Earlier studies from this laboratory likewise revealed the greater sensitivity to dietary fat of skin tumors as contrasted with subcutaneous sarcomas (2, 4, 6). The possibility remained, however, that an effect of fat might become evident on the formation of subcutaneous sarcomas if merely borderline amounts of carcinogen were injected (14, 18). Accordingly, mice fed high or low fat diets were injected subcutaneously with only 20 μgm. of methylcholanthrene in corn oil. The effect of dietary fat was also tested on the development of spontaneous breast tumors in rats, of tumors of the submaxillary gland, and of induced subcutaneous sarcomas (Table III).

The mice receiving the 20 μgm. of methylcholanthrene subcutaneously showed essentially the same incidence of tumors on the diet high in fat as on the low fat control diet (16 and 17 per cent), even though the experimental conditions were presumably ideal

TABLE III: THE INSENSITIVITY OF CERTAIN TYPES OF TUMORS TO DIETARY FAT

| Group | Type of tumor | Methylcholanthrene injected, mgm. | Fat * added to diet, per cent | Effective total | Tumor formation: 5 mos., per cent | 7 mos., per cent | 10 mos., per cent | 30 mos., per cent |
|---|---|---|---|---|---|---|---|---|
| 1 | Induced subcutaneous sarcoma (mice) | 0.02 | 0 | 23 | 9 | 17 | — | — |
| 2 | " " " " | 0.02 | 15 | 30 | 13 | 16 | — | — |
| 3 | Spontaneous breast carcinoma (rats) | 0 | 0 | 33 | — | 0 | 3 | 9 |
| 4 | " " " " | 0 | 30 | 32 | — | 0 | 0 | 6 |
| 5 | Submaxillary gland tumor | 1 | 0 | 5 | 0 | 40 | 40 | — |
| 6 | " " " | 1 | 30 | 5 | 0 | 40 | 40 | — |
| 7 | Induced subcutaneous sarcoma (rats) | 1 | 0 | 8 | 25 | 62 | — | — |
| 8 | " " " " | 1 | 30 | 8 | 38 | 62 | — | — |
| 9 | Induced subcutaneous sarcoma (rats) | 2 | 0 | 6 | 16 | 50 | 83 | — |
| 10 | " " " " | 2 | 30 | 7 | 43 | 57 | 85 | — |

* Hydrogenated vegetable oil, primex.

for demonstrating any accelerating influence that might exist. The presence of 30 per cent of fat in the diet also failed to affect the incidence of spontaneous mammary carcinomas, of induced submaxillary gland tumors, or of induced subcutaneous sarcomas in the rat.

## Attempts to Explain the Action of Fat

*Local and systemic effects.*—The greasy fur of mice fed a high fat diet suggested that the increased tumor incidence observed could have been due in part to a local action of the fat upon the skin. Such an effect was demonstrated when applications of oil increased the rate of formation of tumors due to ultraviolet irradiation (13), or to the carcinogenic hydrocarbons (4, 6, 11, 20). In the present experiments, local applications also increased the total number of tumors produced. The carcinogen was applied twice weekly for the first 2 months of the experiment, and either oleic acid, potassium oleate solution, cottonseed oil, or a 5 per cent solution of cholesterol in cottonseed oil was applied at the site of carcinogenesis on days when the hydrocarbon was not painted. A definite increase in tumor incidence resulted from the local applications: 41 to 59 per cent of the treated animals had tumors at the end of 5 months, as compared to 26 per cent in the control group, which received no applications of oil (Table IV). These results suggested that much of the tumor-promoting activity of dietary fat was exerted locally. However, some fat, no doubt, was absorbed through the skin and the animals were also able to lick off an undetermined amount of the applied oil. Thus enough oil may have entered the general circulation for a systemic effect to be operative as well. The importance of a systemic effect was suggested by the observation that as little as 7 per cent of fat added to a grain mixture containing 5 per cent of fat was sufficient to increase the number of tumors produced (Table II, groups 9 to 13), although no appreciable greasiness of the skins was evident under these conditions. Tannenbaum has also reported increased tumor production in the absence of visible greasiness (18).

Table IV: Tumor Development in the Presence of Oils Applied Locally

(0.2 per cent methylcholanthrene in dioxane, twice weekly, 2 months)

| Oil painted | Effective total | Tumor formation | | | |
|---|---|---|---|---|---|
| | | 2 mos., per cent | 3 mos., per cent | 4 mos., per cent | 5 mos., per cent |
| None | 23 | 0 | 21 | 21 | 26 |
| Oleic acid | 17 | 0 | 23 | 35 | 41 |
| Potassium oleate | 17 | 0 | 17 | 41 | 59 |
| Cottonseed oil | 17 | 0 | 35 | 46 | 59 |
| Cholesterol in cottonseed oil | 19 | 5 | 15 | 37 | 52 |

Purina fox chow was fed to all groups.

Accordingly, mice treated with methylcholanthrene were given emulsified fat incorporated in the drinking water, rather than in the ration. Thus the carcinogenic area of the skin was spared the effects of local contact with dietary fat. The emulsion consisted of 7 per cent of lard suspended in water containing 3 per cent of gum arabic as the emulsifying agent, and was sufficiently stable so that the loss of fat during feeding did not exceed 3 per cent of the total. The animals receiving the emulsion as drink were fed the control diet low in fat, and the incidence of tumors was compared with that in comparable groups on the low fat control diet or on a diet containing 10 per cent of lard. The ingestion of the emulsion varied from week to week, corresponding to intakes of lard that ranged from 7.8 per cent to 21.4 per cent by weight of the total food consumed. Mice fed the emulsion developed more tumors than those on the low fat diet alone, thus confirming the existence of a systemic effect of dietary fat, but the lard fed as an emulsion appeared to be less than half as effective in increasing tumor formation as lard incorporated in the ration. Tumor incidence in the emulsion-fed group was 29 per cent at the end of 6 months, as compared to 18 and 43 per cent respectively in the groups on the low fat control diet and on the diet containing 10 per cent of lard (Table V, groups 1 to 3).

*Metabolic derivatives of fat.*—One suggested explanation of the fat effect was that products of the incomplete metabolism of fat were the agents responsible for the increased tendency toward tumor formation. Accordingly, acetone bodies and a short-chain fatty acid were fed to mice developing skin tumors, and also to rats developing subcutaneous sarcomas or tumors of the submaxillary gland. One group of mice was fed 1 per cent of ethyl acetoacetate incorporated in the low fat control diet, while 2 per cent of acetone was given in the drinking water. A second group was fed a diet containing 10 per cent of coconut oil plus 0.3 per cent of butyric acid. Tumors were induced by the local application of 0.2 per cent of methylcholanthrene in dioxane twice weekly for 2 months, and the incidence of tumors was compared with that in groups on the control diet and on a diet containing 10 per cent of coconut oil. As usual the addition of fat increased the incidence of tumors appreciably (Table VI), but the presence of butyric acid failed to augment this increase: 66 *versus* 63 per cent respectively. Furthermore, the group receiving the low fat control diet plus added acetone bodies de-

TABLE V: RELATION BETWEEN CALORIC INTAKE AND THE INCIDENCE OF TUMORS IN MICE ON VARIOUS DIETS

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

| Diet | Effective total | Cal./25 gm. mouse/day | Tumor formation: 4 mos., per cent | Tumor formation: 6 mos., per cent |
|---|---|---|---|---|
| SERIES A | | | | |
| 1. Low fat control | 22 | 13.5 | 4 | 18 |
| 2. Control + lard emulsion | 25 | 14.7 | 16 | 29 |
| 3. 10% lard | 23 | 15.7 | 30 | 43 |
| SERIES B | | | | |
| 4. Low fat control | 23 | 10.8 | 4 | 4 |
| 5. 10% primex | 22 | 11.1 | 4 | 22 |
| 6. 10% primex + riboflavin | 25 | 12.1 | 40 | 50 |
| SERIES C | | | | |
| 7. Low fat control | 22 | 13.5 | 4 | 18 |
| 8. 10% lard + 7% casein | 21 | 15.3 | 28 | 43 |
| 9. 10% lard (m.p. > 37° C.) | 23 | 15.7 | 30 | 43 |
| 10. 10% triprimex * | 22 | 16.6 | 27 | 41 |
| 11. 10% lard (m.p. < 37° C.) | 24 | 17.6 | 25 | 37 |
| SERIES D | | | | |
| 12. Low fat control (♀) | 17 | 13.0 | 0 | 6 |
| 13. Low fat control (♂) | 14 | 13.4 | 14 | 14 |
| 14. 15% primex † (restricted) | 20 | 13.1 | 10 | 15 |
| 15. 10% primex + riboflavin | 20 | 14.6 | 10 | 30 |
| 16. 15% primex | 22 | 17.2 | 27 | 32 |
| 17. 10% primex | 18 | 17.9 | 11 | 28 |
| 18. Semisynthetic with cooked starch | 21 | 10.8 | 9 | 19 |
| 19. Semisynthetic with glucose | 23 | 12.1 | 22 | 30 |

* Synthetic triglycerides of fatty acids from hydrogenated vegetable oil.
† Hydrogenated vegetable oil.

TABLE VI: THE EFFECT OF METABOLIC DERIVATIVES OF FAT ON THE FORMATION OF SKIN TUMORS IN MICE

(0.2 per cent methylcholanthrene, twice weekly, 2 months)

| Diet | Effective total | Tumor formation: 2 mos., per cent | Tumor formation: 3 mos., per cent | Tumor formation: 5 mos., per cent |
|---|---|---|---|---|
| Low fat control | 21 | 0 | 28 | 33 |
| Low fat control + acetone bodies * | 23 | 0 | 17 | 26 |
| 10% coconut oil | 24 | 4 | 58 | 66 |
| 10% coconut oil + 0.3% butyric acid | 24 | 8 | 54 | 63 |

* 1 per cent ethyl acetoacetate in diet; 2 per cent acetone in drinking water.

veloped essentially the same percentage of tumors as the control group itself: 26 *versus* 33 per cent.

The presence of 15 per cent of sodium butyrate in the diet of rats injected with 2 mgm. of methylcholanthrene failed to increase the incidence of subcutaneous sarcomas. Similarly, the incidence of tumors arising in the submaxillary gland of rats after the insertion of 1 mgm. of methylcholanthrene into this organ was likewise unaffected by feeding a combination of acetone bodies: 1 per cent of ethyl acetoacetate and 5 per cent of sodium butyrate in the diet, and 3 per cent of acetone in the drinking water. Hence it appeared unlikely that dietary fat increased tumor incidence through the medium of the acetone bodies.

*Riboflavin and protein.*—The riboflavin requirement of growing rats is reported to be increased on diets high in fat (8), while an inadequate intake of riboflavin tends to promote the incidence of tumors in rats fed *p*-dimethylaminoazobenzene (5, 9). The possibility therefore existed that the increased incidence of tumors on the high fat diets was due in part to an effective riboflavin deficiency brought about by the extra fat in the diet. Accordingly, 10 mgm. of riboflavin were added per kg. of a typical high fat diet, containing 10 per cent of hydrogenated vegetable oil in addition to the 5 per cent of fat contained in the basal ingredients. When this diet was fed to groups of mice receiving the usual carcinogenic treatment, the incidence of tumors was not only as high, but in some series it was actually higher than in comparable groups of animals receiving the same diet without the added riboflavin (Table V, groups 4, 5, 6, 13, 15, and 17). In a similar manner it was shown that additional protein failed to counteract the tumor-promoting action of the fat (Table V, groups 7 to 11).

*Caloric intake.*—In line with earlier reports (3, 10, 12, 15, 16), Tannenbaum (17) recently observed that more tumors developed in full-fed control mice than

in animals fed only two-thirds of the caloric level adequate for growth. This effect of underfeeding was evident in all strains of mice and with all types of tumors studied: spontaneous tumors of the breast and lung, and carcinomas and sarcomas induced respectively by the local application or by the subcutaneous injection of benzpyrene. A similar effect of underincidence of tumors. Accordingly, measurements were made of the amounts of food consumed by the mice on our various experimental diets, and the caloric equivalent calculated on the basis of 4.1, 4.1, and 9.3 calories per gm. of carbohydrate, protein, and fat respectively. The results were expressed as calories consumed per 25 gm. of body weight of mouse per day.

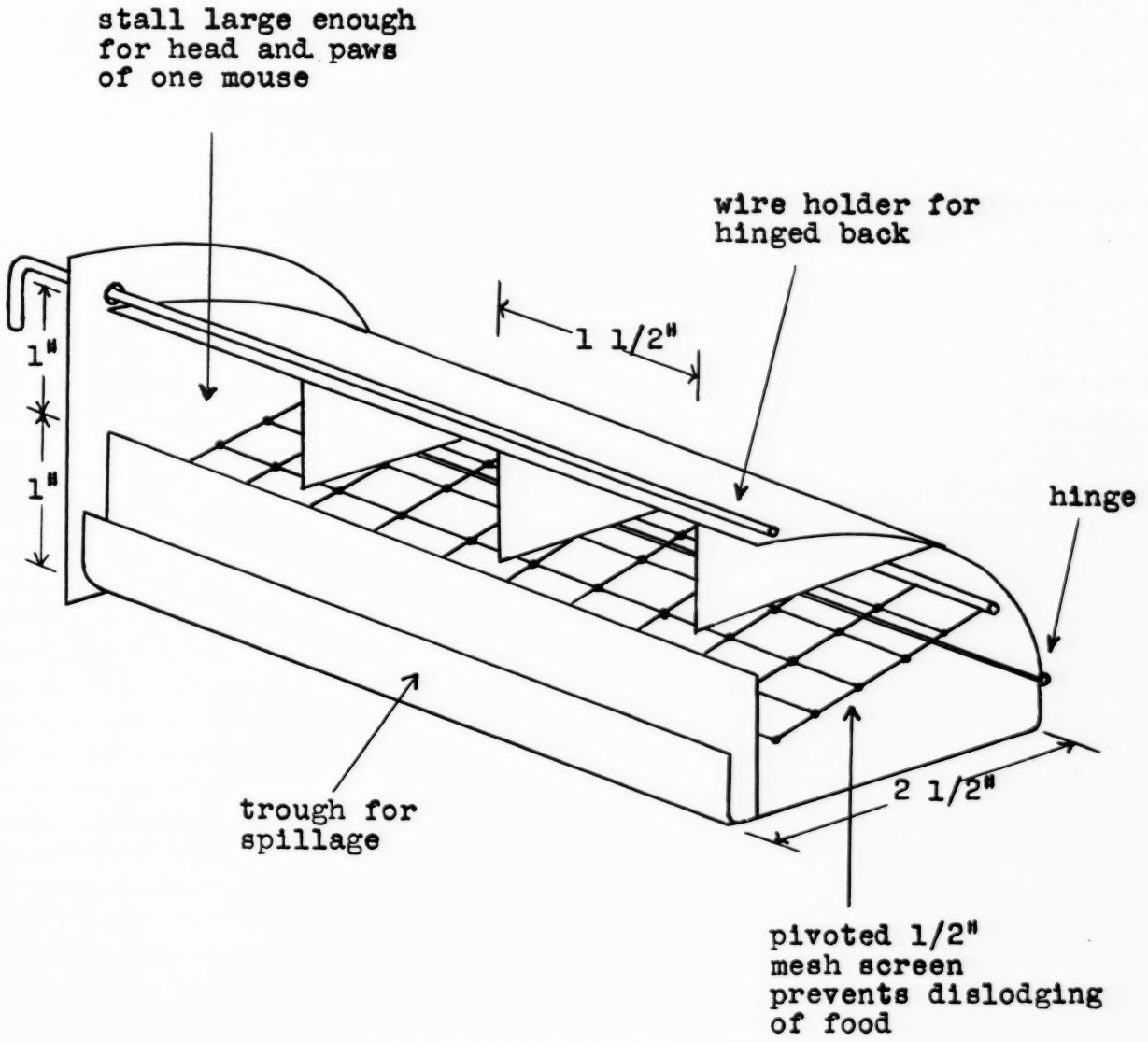



FIG. 1.—Cut-away diagram of mouse feeder.

feeding has been reported by Visscher and his associates (19). In one series a reduction in caloric intake by one-third completely prevented the appearance of mammary carcinomas for 16 months although the full-fed control animals exhibited a tumor incidence of 67 per cent at this time.

These pronounced effects of underfeeding suggested that the converse might also be true to some extent; *viz.*, an increased ingestion of calories above an *ad libitum* level of intake might result in an increased

In each series the number of calories consumed *ad libitum* was least in the group on the low fat control diet (Table V), while the mice on the high fat diets consumed from 12 to 30 per cent more calories than the control animals. Within each series there was a rough correspondence between the number of calories ingested and the number of tumors that ultimately appeared. When mice receiving 15 per cent of hydrogenated vegetable oil were restricted in caloric intake to the number consumed by the low fat control group,

the tumor-promoting effect of the fat was definitely reduced (Table V, groups 12, 13, 14, and 16). An effect of calories was also noted in two low fat semi-synthetic diets containing either cooked starch or glucose monohydrate (cerelose) as the source of carbohydrate. The mice receiving starch consumed 10.8 calories per day, and 19 per cent of these animals developed tumors; those on glucose consumed 12.1 calories per day, and the tumor incidence in this group was 30 per cent.

However, the parallel between caloric intake and tumor incidence was not an absolute one. Small increases in caloric intake within a series sometimes accompanied a large increase in tumor incidence (Table V, groups 4, 5, and 6) while a relatively large difference in caloric intake between the control groups of two different series did not result in any appreciable change in the total number of tumors (Table V, groups 4, 12, and 13). It was therefore evident that factors other than caloric intake exerted some influence on the total number of tumors produced. The increases in caloric intake that appeared to affect tumor incidence in the present experiments were usually less than the differences of 33 per cent reported to be necessary to demonstrate the effects of caloric restriction. The association between the high caloric intake on high fat diets and an increased tendency toward tumor formation was therefore interpreted as suggestive rather than completely proved. It is of interest that a similar conclusion emerges from the results of experiments by Tannenbaum (18) if the caloric intakes of his animals on diets high or low in fat are calculated as in the present report.

The relative effectiveness on tumor development of fat *per se,* as distinct from the extra calories ingested in the form of fat, was estimated in two identical series of experiments involving restricted caloric intake. Each series consisted of 4 groups of 30 mice. The males and females of each group were kept in separate cages, and all mice were painted with a 0.2 per cent solution of methylcholanthrene in dioxane twice weekly for 10 weeks. Weighed amounts of food were fed daily to all groups in special containers (Fig. 1) that enabled the animals to get at the food with a minimum of contact, spillage, or contamination with excreta.

Two of the groups were fed a normal amount of food, 12 calories per 25 gm. of mouse per day, while the other 2 groups were restricted to 8 calories per 25 gm. mouse daily. All groups received the same amount of protein, salts, and vitamins, but the amounts of fat fed at each caloric level were varied so that a 25 gm. mouse ingested either 60 or 240 mgm. of corn oil daily. Thus the following variations were achieved: low fat, low calorie; high fat, low calorie; low fat, high calorie; and high fat, high calorie. The composition of the diets and the amounts fed daily are as follows:—

| | Low fat, low cal. | High fat, low cal. | Low fat, high cal. | High fat, high cal. |
|---|---|---|---|---|
| Cooked starch | 1.01 gm. | 0.60 gm. | 1.99 gm. | 1.58 gm. |
| Casein | 0.60 " | 0.60 " | 0.60 " | 0.60 " |
| Brewers' yeast | 0.24 " | 0.24 " | 0.24 " | 0.24 " |
| Corn oil | 0.06 " | 0.24 " | 0.06 " | 0.24 " |
| Wesson salts (21) | 0.12 " | 0.12 " | 0.12 " | 0.12 " |
| Agar | 0.06 " | 0.06 " | 0.06 " | 0.06 " |
| Halibut liver oil | 30 μgm. | 30 μgm. | 30 μgm. | 30 μgm. |
| | 2.09 gm. | 1.86 gm. | 3.07 gm. | 2.84 gm. |

The combined results of the two series (Table VII) indicate definite effects both of fat and of calories. Regardless of the amount of fat fed, the incidence of tumors was much higher on the high calorie diets than on the low calorie diets. At each level of caloric intake the incidence of tumors was somewhat higher on the high fat diet than on the corresponding low fat diet. However, this extra effect of the fat, over and above the caloric effect, may have been merely an ex-

TABLE VII: THE RELATIVE EFFECTS OF FAT AND OF TOTAL CALORIES ON THE DEVELOPMENT OF SKIN TUMORS DUE TO METHYLCHOLANTHRENE

(0.2 per cent solution in dioxane, twice weekly, 10 weeks)

| | Total mice with tumors/neg. survivors | | | | Effective total | Tumor incidence, per cent |
|---|---|---|---|---|---|---|
| | 3 mos. | 4 mos. | 5 mos. | 6 mos. | | |
| Low fat, low calorie | 0/29 | 0/22 | 0/14 | 0/8 | 29 | 0 |
| High fat, low calorie | 3/25 | 5/19 | 8/9 | 8/9 | 28 | 28 |
| Low fat, high calorie | 15/39 | 26/24 | 29/10 | 29/8 * | 54 | 54 |
| High fat, high calorie | 16/34 | 30/10 | 33/7 | 33/6 | 50 | 66 |

* During the last 2 months the negative survivors did not consume their full allotment of 12 calories per day.

pression of the degree of local contact of fat with the skin, which was minimized by the type of feeder used but was probably not prevented entirely. The greater importance of calories *per se* suggests that much of the systemic or cocarcinogenic activity of dietary fat, if not all, is exerted through the medium of a voluntarily increased intake of calories on diets high in fat.

This, however, does not settle the question of how dietary fat increases the incidence of skin tumors, since the mechanism by which extra calories enhance carcinogenesis is still obscure. It may be that tissues at the periphery of the body ordinarily receive borderline amounts of nutrients, and that the competition among normal cells for food is so high that incipient tumor cells are often suppressed by a kind of starvation. Wicks and Suntzeff (22) recently observed that applications of methylcholanthrene to the skin of mice result in a definite fall in the ratio of total lipid to protein in the epidermis, though not in the whole skin. Under these conditions a general increase in available calories throughout the body might be expected to result in relatively greater improvement in the nutrition of peripheral tumors than of tumor cells in other parts of the body.

## SUMMARY

1. The fatty acids of hydrogenated vegetable oil, prepared free from unsaponifiable matter and resynthesized into triglycerides, had essentially the same tumor-promoting activity as natural fat. The prolonged heating of several natural fats did not greatly alter their tumor-promoting activity.

2. Much of the effect of the dietary fat was exerted locally. The application of oil to the skin increased the total number of tumors produced, while fat fed as an emulsion in the drinking water was only about half as effective in increasing tumors as fat incorporated into the diet.

3. Possible products of fat metabolism, such as acetone, ethyl acetoacetate, and butyric acid, did not appreciably increase incidence of the tumors in either rats or mice. An increased intake of riboflavin and protein failed to diminish the tumor-promoting action of the fat. Increased dietary fat did not increase the incidence of sarcomas in mice caused by the subcutaneous injection of methylcholanthrene, nor of spontaneous breast tumors of rats, nor of tumors induced by the injection of methylcholanthrene either subcutaneously or into the submaxillary gland of the rat.

4. Mice painted with methylcholanthrene consumed 12 to 30 per cent more calories on diets high in fat than on corresponding low fat diets. A rough correspondence was observed between the caloric intakes of mice on the various rations and the numbers of tumors that ultimately appeared. On an equivalent caloric intake, the incidence of tumors in mice on the high and low fat diets was more nearly equal. It is suggested that at least part of the tumor-promoting action of fat is due to an accompanying increased consumption of calories.

## REFERENCES

1. Baumann, C. A., and Rusch, H. P. Effect of Diet on Tumors Induced by Ultraviolet Light. Am. J. Cancer, **35**:213-221. 1939.
2. Baumann, C. A., Jacobi, H. P., and Rusch, H. P. The Effect of Diet on Experimental Tumor Production. Am. J. Hyg., **30**:1-6. 1939.
3. Bischoff, F., and Long, M. L. The Influence of Calories *per se* upon the Growth of Sarcoma 180. Am. J. Cancer, **32**:418-421. 1938.
4. Jacobi, H. P., and Baumann, C. A. The Effect of Fat on Tumor Formation. Am. J. Cancer, **39**:338-342. 1940.
5. Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P. Partial Protection of Rats by Riboflavin with Casein against Liver Cancer Caused by Dimethylaminobenzene. Science, **93**:308-310. 1941.
6. Lavik, P. S., and Baumann, C. A. Dietary Fat and Tumor Formation. Cancer Research, **1**:181-187. 1941.
7. Lavik, P. S. Dietary Factors Affecting Tumor Formation. Doctor of Philosophy Thesis, University of Wisconsin. 1943.
8. Mannering, G. J., Lipton, M. A., and Elvehjem, C. A. Relation of Dietary Fat to Riboflavin Requirement of Growing Rats. Proc. Soc. Exper. Biol. & Med., **46**: 100-104. 1941.
9. Miller, J. A., Miner, D. L., Rusch, H. P., and Baumann, C. A. Diet and Hepatic Tumor Formation. Cancer Research, **1**:699-708. 1941.
10. Moreschi, C. Beziehungen zwischen Ernährung und Tumorwachstum. Ztschr. f. Immunitätsforsch. u. exper. Therap., **2**:651-675. 1909.
11. Morton, J. J., and Mider, G. B. Effect of Petroleum Ether Extract of Mouse Carcasses on Skin Tumor Formation in C57 Black Mice. Pub. Health Rep., **55**:670-676. 1940.
12. Rous, P. The Influence of Diet on Transplanted and Spontaneous Mouse Tumors. J. Exper. Med., **20**:433-451. 1914.
13. Rusch, H. P., Baumann, C. A., and Kline, B. E. Effect of Local Applications on Development of Ultraviolet Tumors. Proc. Soc. Exper. Biol. & Med., **42**:508-512. 1939.
14. Sall, R. D., and Shear, H. J. Studies in Carcinogenesis. XII. Effect of the Basic Fraction of Creosote Oil on the Production of Tumors in Mice by Chemical Carcinogens. J. Nat. Cancer Inst., **1**:45-55. 1940.
15. Sugiura, K., and Benedict, S. R. The Influence of Insufficient Diets upon Tumor Recurrence and Growth in Rats and Mice. J. Cancer Research, **10**:309-318. 1926.
16. Tannenbaum, A. The Initiation and Growth of Tumors. Introduction. I. Effects of Underfeeding. Am. J. Cancer, **38**:335-350. 1940.
17. Tannenbaum, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction *per se*. Cancer Research, **2**:460-467. 1942.

18. Tannenbaum, A. The Genesis and Growth of Tumors. III. Effects of a High-Fat Diet. Cancer Research, **2**: 468-475. 1942.
19. Visscher, M. B., Ball, Z. B., Barnes, R. H., and Sivertsen, I. The Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48-55. 1942.
20. Watson, A. F., and Mellanby, E. Tar Cancer in Mice. II. The Condition of the Skin When Modified by External Treatment or Diet, as a Factor in Influencing the Cancerous Reaction. Brit. J. Exper. Path., **11**:311-322. 1930.
21. Wesson, L. G. A Modification of the Osborne-Mendel Salt Mixture Containing Only Inorganic Constituents. Science, **75**:339-340. 1932.
22. Wicks, L. F., and Suntzeff, V. Reduction of Total Lipid-Protein Nitrogen Ratio of Mouse Epidermis by a Single Application of Methylcholanthrene. J. Nat. Cancer Inst., **3**:221-226. 1942.

# Spontaneous Testicular Tumors in Mice*

W. U. Gardner, Ph.D.

*(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)*

(Received for publication June 7, 1943)

Testicular interstitial cell tumors have appeared in estrogen-treated mice of at least three different inbred strains (1, 2, 11, 12, 13, 21), rarely in those of other strains treated in a comparable manner (2, 11, 21). Spontaneous testicular tumors were not described in those inbred strains that acquired such tumors subsequent to prolonged treatment with estrogens. In fact such tumors occur rarely in mice. Among 19,000 mice autopsied, of which approximately one-half were males, only 28 primary tumors of the testes were found (27). All except one of the 28 mice bearing a testicular tumor were from one family or its hybrids, an observation indicating a tendency to hereditary predisposition. Only one of the 28 tumors consisted of the interstitial cells of Leydig; the others were spindle cell sarcomas or composed "of cells closely resembling the epithelium of the seminiferous tubules arranged in alveolar structure."

Mice of the Strong A strain, when adequately treated with estrogens, show hypertrophy of the Leydig cells (1, 7, 8, 13) and finally testicular tumors composed largely of such cells. Estrogen-treated mice of several other inbred strains failed to show a hypertrophy of the Leydig cells, and testicular tumors rarely or never appeared. The tendency for such tumors to occur in estrogen-treated animals was thus strain-limited.

The question arises whether or not estrogen merely results in the attainment of potentialities for testicular neoplasia that are usually not realized. The appearance of spontaneous tumors among mice that would acquire a high incidence of tumors if estrogens were injected is reported here.

## MATERIALS AND METHODS

In order to determine to some extent the mode of transmission of the tendency to develop testicular tumors, mice of the A strain were hybridized with mice of either the C57 or the C3H strain (24, 25).[1] Because both male and female mice of the A strain were used four groups of hybrids were obtained as shown in Table I. Both males and females from the A strain transmitted the tendency to acquire testicular tumors to their estrogen-treated first generation hybrids, obtained by matings with mice of the C3H and C57 strains. The studies to be reported here are limited, however, to the untreated control mice of each group. A total of 61 untreated hybrid male mice from the four groups were observed throughout their lives. Twenty-four male mice of the A strain were studied also. All mice were maintained on Purina fox chow and water. The males were kept in cages with one or more males and females until the latter died. From that time on 3 to 5 males were kept together in one compartment. When the conditions of the animals indicated that death was imminent they were killed and an autopsy was performed. If the testes were grossly abnormal they were fixed in Bouin's fluid, sectioned, and stained with hematoxylin and triosin.

TABLE I: THE INCIDENCE OF TESTICULAR TUMORS AMONG UNTREATED MICE OF THE A STRAIN AND OF HYBRIDS

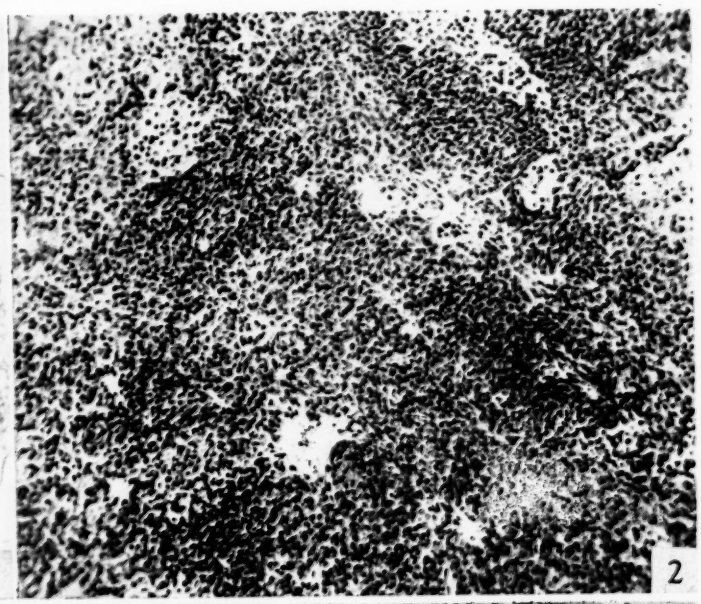
| Group | Parental strains | Number of mice | Average age, days | Number of tumors | Age at death of mice with tumors, days |
|---|---|---|---|---|---|
| AC1 | A ♀ × C3H ♂ | 15 | 737 | — | — |
| AC2 | C3H ♀ × A ♂ | 10 | 781 | 1 | 952 |
| A71 | A ♀ × C57 ♂ | 16 | 823 | — | — |
| A72 | C57 ♀ × A ♂ | 20 | 752 | 2 | 810–816 |
| A | A ♀ × A ♂ | 24 | 514 | — | — |

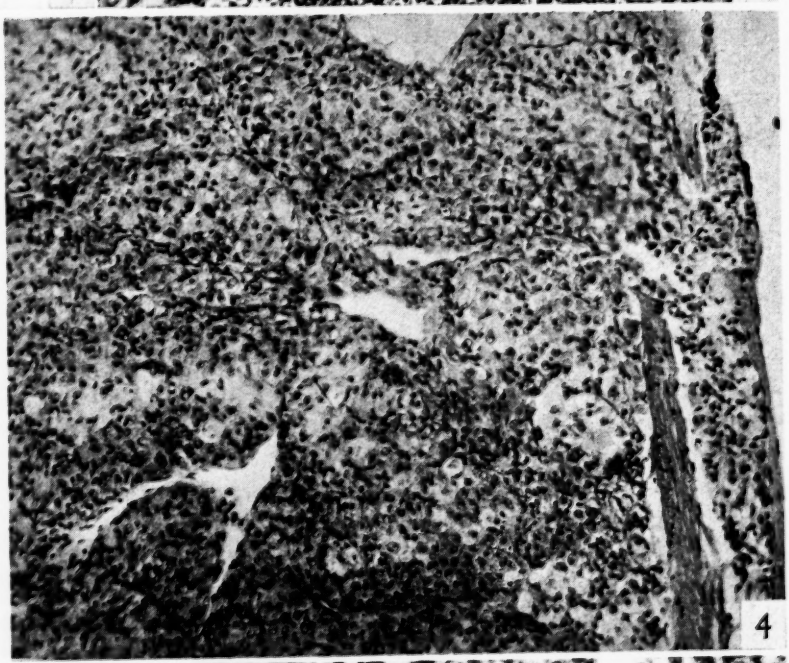
## OBSERVATIONS

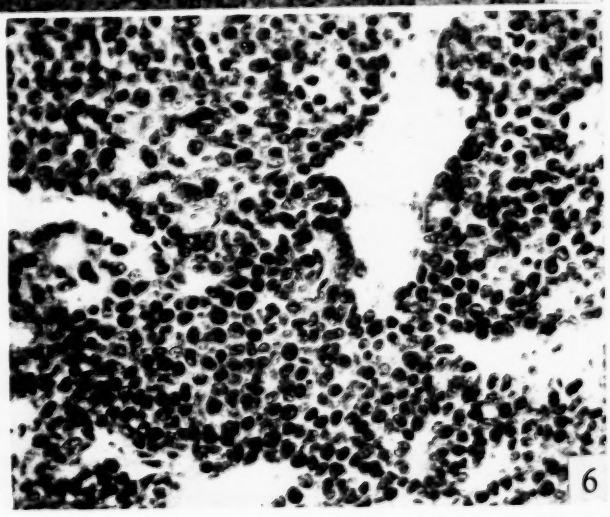
In 3 of the 61 male mice from two of the four hybrid groups primary testicular tumors were discovered at autopsy. The tumorous testis of one of the mice of the A72 group killed when 810 days old was approximately twice the size of a normal testis (Fig. 1) and measured 1.2 cm. × 0.8 cm. The tumorous testis had a smooth surface and was of mottled yellow and brown color. The nontumorous testis was of approximately normal size and pale gray. The seminal vesicles and prostate of this mouse were of approximately normal size but contained no secretion; they did not differ from those of a number of other old mice of this group that had no testicular tumors.

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Anna Fuller Fund.

[1] The mice from these strains were supplied by Dr. L. C. Strong.







FIGS. 1-6

The tumor was composed of two types of tissue. One part contained small cells, not unlike some nonvacuolated interstitial cells, and many macrophages, some containing a brown pigment similar to that observed frequently in the testicular interstitial tissues of estrogen-treated mice (Fig. 2). Another part, constituting approximately half the tumor, was composed of small round cells and cells resembling fibroblasts. Mitotic figures were abundant among the interstitial cells that formed the first mentioned portion of the tumor. Vestiges of seminiferous tubules were found near the periphery (Fig. 1). This tumor resembled more closely those neoplasias described as seminomas than the usual interstitial cell tumors. The nontumorous testis showed a few "brown cells" and small glandular interstitial cells with a granular cytoplasm. It appeared that Leydig cells were smaller and fewer than in the testis of other old mice.

The second testicular tumor in a mouse of the A72 group was small, only slightly enlarging the testis (Fig. 3). It was detected at autopsy as a hyperemic, yellow body in the proximal half of the testis. Histologically it consisted of large glandular cells with either a clear, vacuolated or a granular cytoplasm, and large vesicular nuclei containing one or two large nucleoli (Fig. 4). This tumor was histologically much like those described previously in estrogen-treated mice of the A, C, and JK strains. The tumor occupied a large part of the testis; the tubules remaining at the periphery contained few spermatids or spermatocytes. The nontumorous testis was similar to those of other old mice of the group, only a few small granular Leydig cells and "brown" cells representing the interstitial elements of interest at this time. The seminal vesicles were very large and distended with secretion.

The tumor arising in the mouse of the AC2 group occupied approximately half the testis and was observed upon gross examination in its mediastinal portion. The area of the tumorous nodule in which the tubules were entirely destroyed was extremely hyperplastic, and surrounded by a more fibrous tissue that widely separated the seminiferous tubules (Fig. 5). This tumor was composed of small cells with scanty cytoplasm and nuclei containing peripheral chromatin aggregates and one or two small nucleoli (Fig. 6). The growth was not sharply circumscribed; at the periphery it encompassed the more or less atrophic seminiferous tubules (Fig. 5). This tumor was composed of cells similar to those of the "third generation" described by Hooker and Pfeiffer (13) in their estrogen-treated mice.

Hyperplasias or tumors of the interstitial cells sufficiently large to be detected upon gross examination were not observed in any testes of the mice of the A strain.

## DISCUSSION

It has been observed by several investigators that estrogen-treated mice of some inbred strains acquire interstitial cell tumors of the testis. Although such tumors may occur also among untreated animals of some families of mice, as indicated by the work of Slye, Holmes, and Wells (22), no evidence has been reported that they will occur in mice of those strains or groups in which they appear when estrogens are injected. Testicular tumors have not been described among untreated mice of the A, C, and JK strains kept under the same conditions as those animals that, when given estrogen, acquired such tumors (1, 11, 21). The hybrid mice, however, survived 200 to 300 days longer on the average than their A strain parents (Table I), and it may be that the increased longevity afforded an adequate period for the materialization of inherent tendencies that might be elicited precociously in estrogen-treated animals. Spontaneous testicular interstitial cell tumors tend to appear among aged animals (see reference 27 for literature).

The recent experiments on the induction of testicular interstitial cell tumors in inbred mice thus indicate that two influences are essential for the inception of such neoplasia; namely, the humoral environment maintained by estrogen injections and some genetic predisposition. The present observations reveal that the more vigorous and longer lived hybrid mice with an adequate genetic predisposition may acquire testicu-

## DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Cross-section of testicular tumor of a mouse of the A72 group showing its gross structure. A few atrophic seminiferous tubules at the right and upper part of the photograph. Mag. × 7.5.

FIG. 2.—Approximately one-half of the tumor shown in Fig. 1 was composed of small cells resembling fibroblasts, only a few of which were similar to glandular interstitial cells. Mag. × 100.

FIG. 3.—Longitudinal section through testis of a mouse of the A72 group showing an interstitial cell tumor that occupied about half the testis. Mag. × 7.5.

FIG. 4.—Higher power view of tumor from Fig. 3. Composed of large cells with either a pale or granular eosinophilic cytoplasm. The tumor has invaded the tunica albuginea at the right. Mag. × 100.

FIG. 5.—Area surrounding the tumorous nodule in the testis of the mouse of the AC2 group. The seminiferous tubules are separated by small cells. Mag. × 100.

FIG. 6. Higher power view of tumorous nodule from Fig. 5. Its cells, which occupied approximately one-third of the testis, resembled those found in some of the Leydig cell tumors and showed numerous mitotic figures. Mag. × 210.

lar tumors in the absence of extrinsic hormonal factors. Testicular neoplasias have not been observed among hybrid mice obtained by crossing mice of other inbred strains, although these animals were kept under similar conditions (9, 10).

Since the testicular interstitial cells atrophy after removal of the hypophysis their morphological integrity apparently depends upon the hypophysial hormones. It is probable that the estrogens act on the pituitary gland (14), the latter being more directly associated with the initiation of the hypertrophic and finally the neoplastic changes, a theory substantiated by the observation that a gonadotropic preparation, pregnant mare serum, induced local hypertrophy of the interstitial cells of mice of the A strain (18). The inherited factors that limit the testicular tumors to mice of certain strains may therefore involve peculiarities of either the testicular or hypophysial physiology or both.

The possibility that trauma might play an etiological role in testicular neoplasia in mice has been suggested (22) and should not be overlooked. Inguinal and scrotal wounds occur frequently during fights, especially between male mice. That lesions might have occurred in the testes of the mice that spontaneously developed testicular tumors is possible. At the time of death, however, evidence of such lesions was lacking. As far as is known experiments have not been undertaken in which controlled lesions have been made in the testes of animals of these strains. Maximow (15), however, observed abundant mitotic figures among the interstitial cells in the vicinity of sterile testicular lesions, indicating that trauma may incite proliferation. Ovarian grafts placed in the testes of mice of other strains may elicit an increase of interstitial cells among the damaged tubules that accompany such precedures (6).

The size and structure of the cellular elements of the spontaneous testicular neoplasia in these mice varied greatly, not only from one tumor to another but between different parts of the same growth. In some tumors islands of hematopoietic tissue have been observed (11). Interstitial cells have been described as arising from lymphocytes (16, 17), or more frequently from cells indistinguishable from fibroblasts (15, 17, 19, 26, 28). The undifferentiated cells from which the Leydig cells may arise may undergo changes in several directions. The glandular cells, hematopoietic cells, and macrophages may all have a common origin from undifferentiated intertubular elements.

The presence of "brown pigmented" cells in the testes of the old mice with and without testicular tumors, and among the peripheral cells of the tumors, has been mentioned previously (1, 3, 4, 5, 8, 11, 13). Such cells have been described in the testes of birds and mammals by several investigators and are found usually when there is evidence to indicate a regression of the testis (see reference 20 for review). In old mice the number of interstitial cells decreases as age advances (23), and as observed in the present study the brown pigment was found in the interstitial cells of old mice.

## SUMMARY AND CONCLUSIONS

1. Testicular tumors arose in 3 of 61 untreated hybrid mice obtained by mating the animals of the A with those of the C3H and C57 strains. Two of these were interstitial cell tumors and histologically resembled those arising in estrogen-treated mice of similar origin. The third, composed of fibroblastic cells and small round cells, was probably an interstitial cell tumor but resembled a seminoma rather than the usual interstitial cell tumor.

2. Testicular tumors were not observed in untreated mice of the A strain, but these animals survived on the average 200 to 300 days less than their $F_1$ hybrids.

3. It is possible that in mice of certain strains estrogens may act to reveal or augment tendencies to acquire testicular interstitial cell tumors that otherwise would not be attained during the usual life span.

## REFERENCES

1. Bonser, G. M. Malignant Tumours of the Interstitial Cells of the Testis in Strong A Mice Treated with Triphenylethylene. J. Path. & Bact., **54**:149-154. 1942.
2. Bonser, G. M., and Robson, J. M. The Effects of Prolonged Oestrogen Administration upon Male Mice of Various Strains: Development of Testicular Tumours in the Strong A Strain. J. Path. & Bact., **51**:9-22. 1940.
3. Burrows, H. Changes Induced in the Interstitial Tissue of the Testis of the Mouse by Certain Oestrogens. J. Path. & Bact., **41**:218-219. 1935.
4. Burrows, H. A Comparison of the Changes Induced by Some Pure Oestrogenic Compounds in the Mammae and Testes of Mice. J. Path. & Bact., **42**:161-168. 1936.
5. Burrows, H. Acquired Resistance to Oestrone in a Male Mouse. J. Path. & Bact., **44**:699-701. 1937.
6. Gardner, W. U. The Effect of Ovarian Hormones and Ovarian Grafts upon the Mammary Glands of Male Mice. Endocrinology, **19**:656-667. 1935.
7. Gardner, W. U. Interstitial Cell Hypertrophy in the Testes of Mice Receiving Estrogenic Hormones. Anat. Rec. (suppl.), **67**:49. 1936.
8. Gardner, W. U. Hypertrophy of Interstitial Cells in the Testes of Mice Receiving Estrogenic Hormones. Anat. Rec., **68**:339-347. 1937.
9. Gardner, W. U. The Effect of Estrogen on the Incidence of Mammary and Pituitary Tumors in Hybrid Mice. Cancer Research, **1**:345-358. 1941.
10. Gardner, W. U., and Strong, L. C. Strain-Limited Development of Tumors of the Pituitary Gland in Mice Receiving Estrogens. Yale J. Biol. & Med., **12**:543-548. 1940.

11. Gardner, W. U. Testicular Tumors in Mice of Several Strains Receiving Triphenylethylene. Cancer Research, **3**: 92-99. 1943.
12. Hooker, C. W., Gardner, W. U., and Pfeiffer, C. A. Testicular Tumors in Mice Receiving Estrogens. J.A.M.A., **115**:443-445. 1940.
13. Hooker, C. W., and Pfeiffer, C. A. The Morphology and Development of Testicular Tumors in Mice of the A Strain Receiving Estrogens. Cancer Research, **2**:759-769. 1942.
14. Lane, C. E. Some Influences of Oestrin on the Hypophyseal-Gonad Complex of the Immature Female Rat. Am. J. Physiol., **110**:681-685. 1935.
15. Maximow, A. Die histologischen Vorgänge bei der Heilung von Hodenverletzungen und die Regenerationsfähigkeit des Hodengewebes. Beitr. z. path. Anat., **26**:230-319. 1899.
16. Nonidez, J. F. Studies on the Gonads of the Fowl. I. Hematopoietic Processes in the Gonads of Embryos and Mature Birds. Am. J. Anat., **28**:81-113. 1920.
17. Nonidez, J. F. Studies on the Gonads of the Fowl. IV. The Intertubular Tissues of the Testis in Normal and Hen-Feathered Cocks. Am. J. Anat., **34**:359-392. 1924.
18. Pfeiffer, C. A., and Hooker, C. W. The Effects of Pregnant Mare Serum upon the Course of Development of Testicular Tumors in Mice of the A Strain. Cancer Research, **3**:124-125. 1943.
19. Rasmussen, A. T. Seasonal Changes in the Interstitial Cells of the Testis in the Woodchuck (*Marmata monax*). Am. J. Anat., **22**:475-515. 1917.
20. Rasmussen, A. T. Interstitial Cells of the Testis. Special Cytology. Edited by E. V. Cowdry. Vol. II. 1928, pp. 1211-1256.
21. Shimkin, M. B., Grady, H. G., and Andervont, H. B. Induction of Testicular Tumors and Other Effects of Stilbestrol-Cholesterol Pellets in Strain C Mice. J. Nat. Cancer Inst., **2**:65-80. 1941.
22. Slye, M., Holmes, H. F., and Wells, H. G. Primary Spontaneous Tumors of the Testicle and Seminal Vesicle in Mice and Other Animals. XII. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice. J. Cancer Research, **4**:207-228. 1919.
23. Stieve, H. Untersuchungen über die Wechselbeziehungen zwischen Gesamtkörper und Keimdrüsen. II. Beobachtung und Versuche an männlichen Hausmäusen und an männlichen Feldmäusen, zugleich ein weiterer Beitrag zur Zwischenzellenfrage. Arch. f. mikr. Anat., **99**:390-570. 1923.
24. Strong, L. C. The Origin of the JK Strain of Inbred Mice. J. Hered., **28**:40-42. 1937.
25. Strong, L. C. The Origin of Some Inbred Mice. Cancer Research, **2**:531-539. 1942.
26. Wagner, K. Sind die Zwischenzellen des Säugetierhodens Drüsenzellen? Ein Beitrag zur Zytology and Zytogenese. Biologia Generalis, **1**:22-51. 1925.
27. Warren, S., and Olshausen, K. W. Interstitial Cell Growths of the Testicle. Am. J. Path., **19**:307-331. 1943.
28. Whitehead, R. H. The Embryonic Development of the Interstitial Cells of Leydig. Am. J. Anat., **3**:167-182. 1904.

# Testicular Changes Resembling Early Stages in the Development of Interstitial Cell Tumors in Mice of the A Strain after Long-Continued Injections of Pregnant Mare Serum*

Carroll A. Pfeiffer, Ph.D., and Charles W. Hooker, Ph.D.

*(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)*

(Received for publication June 11, 1943)

Administration of estrogens has resulted in the development of interstitial cell tumors of the testis in certain strains of mice (1, 2, 6, 8, 9, 20). In the A strain (9) the first testicular change in response to the estrogen was hypertrophy of the Leydig cells without conspicuous increase in their number. Later the hypertrophied cells disappeared, probably by being phagocytosed. Formation of new Leydig cells then occurred throughout the testis, with a more rapid formation in one or more loci resulting in the appearance of one or more nodules of Leydig cells. Continued growth of the nodules produced gross enlargement of the testis.

A direct action of the estrogen upon the testis does not seem highly probable. Instead, an alteration of the mechanism that normally controls the formation and function of Leydig cells is a somewhat more attractive possibility. Unfortunately, the control of the Leydig cells is at best but poorly understood, although this is generally believed to be one of the functions of the hypophysis. Administration of estrogen has been shown to augment the release of hypophyseal luteinizing hormone (12), a substance that is reported (7) to stimulate Leydig cells. If the action of the estrogen in eliciting testicular tumors is mediated by the hypophysis, long-continued treatment with the appropriate gonadotropin should produce effects upon the testis similar to those that follow treatment with estrogens. The fact that the mouse very soon develops refractoriness to many gonadotropic preparations (17) renders most of these substances useless for the present purpose. On the other hand, pregnant mare serum retains its effectiveness in the mouse over months of treatment (18). Moreover, administration of this substance has resulted in the formation of large numbers of Leydig cells in the testis of the sparrow (19), a species normally possessing few or none of these cells. For these reasons, therefore, the following experiment was conducted to determine whether long-continued administration of pregnant mare serum will produce testicular tumors or any testicular changes simulating any stages in the development of testicular tumors of estrogen-treated mice.

* This study was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research; the National Research Council, Committee for Research in Problems of Sex; The Anna Fuller Fund; The International Cancer Research Foundation; and the Fluid Research Fund of Yale University School of Medicine. The pregnant mare serum (gonadin) was supplied by Cutter Laboratories, through the courtesy of Drs. D. H. Wonder and C. Parham.

## EXPERIMENTAL

Nineteen male mice of the A strain of Strong (21) were given daily injections of pregnant mare serum (PMS). In one group of 10 the daily dose was 50 I.U., and the first injection was given when the animals were 35 days old. The second group of 9 animals was given daily injections of 20 I.U. from the 10th until the 35th day of life; thereafter the daily dose in this group was 50 I.U. To determine whether there were early changes, one animal from each group was autopsied after 30 days and another after 40 days of treatment. The remaining animals were treated continuously until death occurred or appeared imminent, permitting a period of uninterrupted daily treatment ranging from 300 to 550 days. Laparotomies at intervals allowed examination of the macroscopic condition of the reproductive system. At necropsy the testes, accessory glands of reproduction, regional lymph nodes, kidneys, and adrenal glands were preserved in Bouin's picroformol. These tissues were embedded in paraffin, sectioned at 8 micra, and stained with Ehrlich's hematoxylin and triosin. The diet consisted of Purina fox chow and fresh water, both of which were continuously available.

## OBSERVATIONS

By the end of one month of treatment the testes had enlarged slightly, and a very slow increase in size continued through 100 days of treatment. Thereafter,

for a time, the testes diminished in size, with the diminution becoming conspicuous by the 180th day. The reduced size of the testis persisted to the end of 12 months of treatment. Thereafter the normal size was regained and even somewhat exceeded. The superficial blood vessels of the testis became much less convoluted after 100 days of treatment, and the tunica albuginea appeared to be rather taut. At no time did the testes acquire the yellow or brown color exhibited by males of this strain receiving estrogens (9).

The accessory glands of reproduction (seminal vesicles, prostate glands, and coagulating glands) increased in size almost progressively from the beginning of treatment, and showed no decrease in size or diminished growth even during the period of reduced testicular size. All these glands came to be distended with secretion. The seminal vesicles developed a prickly contour, and frequently exhibited an incipient duplication. The coagulating glands enlarged greatly, became somewhat erect, and in configuration resembled a bunch of bananas. Accessories of this type are characteristic of the Strong C strain of mice and this morphology has been attributed to the action of high levels of androgen (10). Hypertrophy of the preputial glands and the penis was also present.

No conspicuous differences were evident between the animals treated from the 10th and from the 35th day of age, except that a fibrosarcoma developed at the site of injection after 10 months of treatment in 2 of the latter group. The kidneys of the treated animals were enlarged, weighing up to 750 mgm. in some instances. The salivary glands and the harderian glands were much enlarged in the animals treated for more than 14 months. The hypophyses were fairly small in all the older animals.

*Microscopic findings.*—The tissues from 4 of the animals had undergone too much postmortem change to permit histologic study. In the remaining 15 animals the condition of the interstitial cells of the testis ranged from no discernible effect of the treatment to the accumulation of epithelioid cells to form a nodule having a cross sectional area equivalent approximately to that of two tubules cut transversely. No greater change was found, even after 18 months of uninterrupted daily treatment. The extent of the increase in the numbers of interstitial cells seems to depend roughly upon the length of treatment, although no precise correlation was possible. In 10 of the animals the seminiferous tubules appeared not to be affected by the treatment, while in the remaining 5 cases spermatogenesis was impaired to various degrees, in one instance to the point of resembling that usually seen in cryptorchid testes (13). No uniform correlation of tubular damage with duration of treatment or with interstitial cell change was apparent.

The testes of 9 of the treated animals contained discrete nodules of interstitial cells (Figs. 2 and 3), with the nodules ranging from one-third of the cross sectional area of a tubule to twice the area of a tubule. The cells comprising these nodules (Figs. 4 and 5) were approximately the size and shape of normal Leydig cells and resembled cells of the "second generation" in the testicular tumors of estrogen-treated mice of this strain (9). Their cytoplasm stained a dull red with eosin, was characterized by small granules, and contained small vacuoles. The nuclei were perhaps slightly enlarged, often somewhat wrinkled, and usually the chromatin was less dense than in the normal cell. In 5 of the 9 animals many of these nuclei contained eosinophilic inclusion bodies of various sizes, which were of frequent occurrence in cells of the first and second generations in the testicular tumors of estrogen-treated mice. Mitoses were fairly abundant in these cells in one animal treated for 430 days, but were rare or absent in the others.

The nodules of cells were usually fairly sharply localized, although in one instance the cells made up a broad sheet. No histologic feature was seen that would account for the delimitation. The nodules occupied various positions in the testis, the most frequent being eccentric and in the vicinity of the mediastinum testis. Local invasions by these cells were not observed.

In the testes of 4 of the treated animals the interstitial cells appeared to be fairly definitely increased in number but without a sufficient increase to form nodules. These cells were morphologically indistinguishable from normal Leydig cells. The increase in number occurred throughout the testis, but was most evident toward the periphery. No change in either the number or structure of the interstitial cells was evident in the 2 remaining animals. The brown cells, which are abundant in early stages of the development of testicular tumors (9), were seen in the testes of 2 animals, one after 292 and the other after 430 days of treatment. Mast cells were common in the testes of these 2 animals.

As would be expected, the lymph nodes, which are frequently invaded by cells from testicular tumors in estrogen-treated mice of this strain (9), were completely free of the interstitial cells. In a number of the animals the renal and lumbar nodes contained brown cells.

Except in one animal the accessory glands of reproduction exhibited the histologic features that characterize these organs when they are under the influence of androgen. Their lumina were filled with secretion, and their epithelia were tall and contained abundant secretion granules. In the exceptional animal low grade castration changes were evident in these structures. The kidneys were characterized especially

by a heightened glomerular epithelium, such as is seen after administration of androgen. The adrenal glands of one animal exhibited subcapsular hyperplasia and in 2 animals small adrenal tumors resembling those described by Woolley, Fekete, and Little (23) were present. The adrenal glands of the remaining animals were structurally normal.

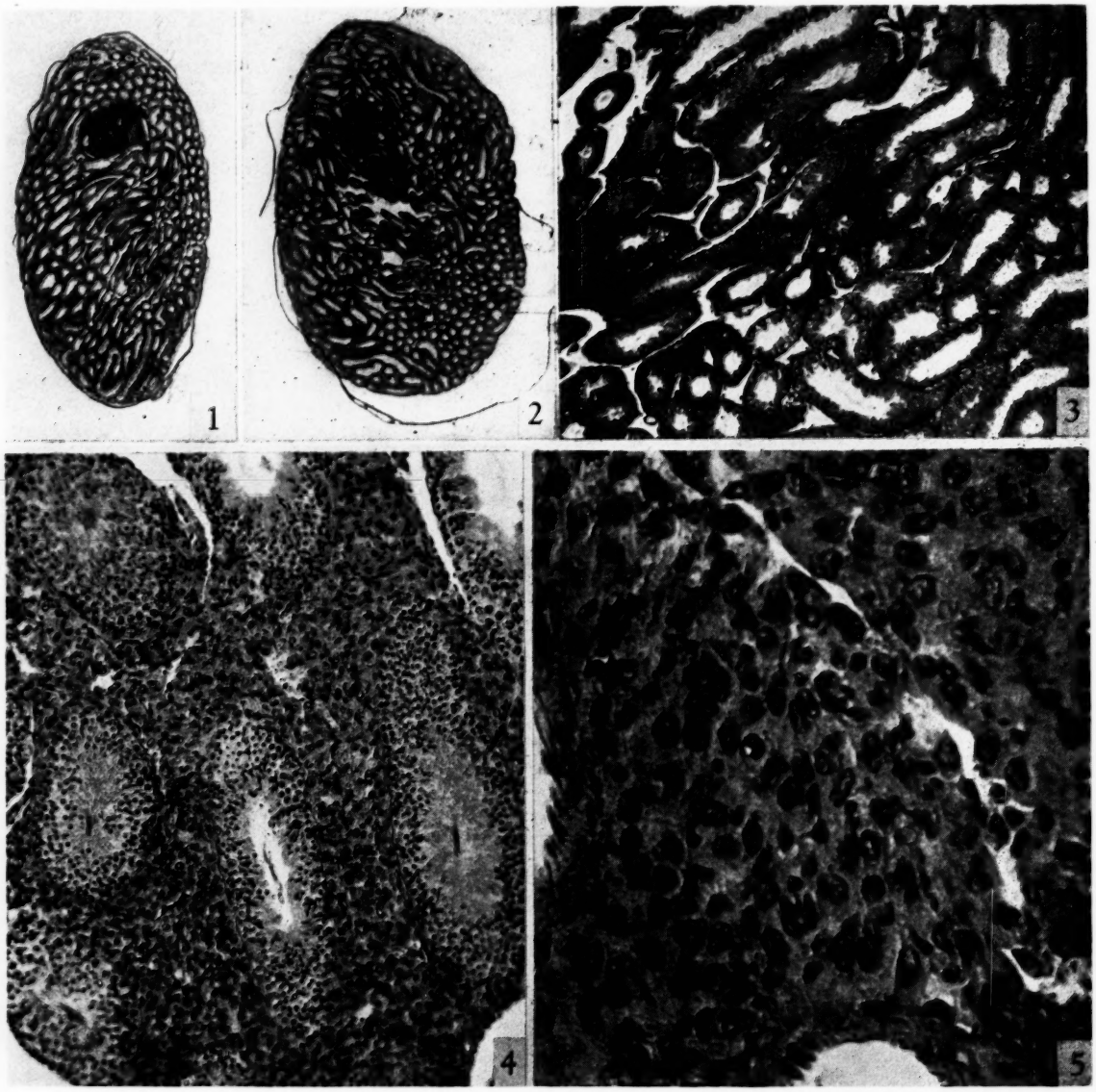



FIG. 1.—Testis from a mouse of the A Strain after 6 months' treatment with 0.25 mgm. stilbestrol weekly. The nodule is composed of interstitial cells and represents an early interstitial cell tumor. Mag. × 8.3.

FIG. 2.—Testis showing accumulations of interstitial cells just above its center. The animal had been given daily injections of 50 I.U. of PMS for 14 months. Nine of 15 animals so treated showed this change. Compare with Fig. 1. Mag. × 8.3.

FIG. 3.—Area of testis from Fig. 1 showing the relatively localized distribution of these accumulations. Mag. × 40.

FIG. 4.—Area of the testis showing the character of the interstitial cells and their relation to the well preserved tubules. Mag. × 110.

FIG. 5.—Nest of interstitial cells from the same testis. These cells are similar both to the interstitial cells of untreated mice and to cells of the "second generation" in testicular tumors in estrogen-treated mice of this strain. Mag. × 415.

## DISCUSSION

The significant fact in this experiment is the essential morphological similarity of the nodules in the testes of animals that had been subjected to prolonged treatment with PMS to the young nodules in the testes of estrogen-treated mice (9). Compare Figs. 1 and 2. The establishment of the nodules in the PMS-treated animals appeared to be essentially progressive, and to have been the consequence of accelerated transformation of mesenchymal or fibroblastic elements into Leydig cells. Thus the two hormones, estrogen and PMS, produced the same end result, with the response to the latter apparently omitting two preliminary stages that are seen in estrogen-treated mice.

The nodules in the PMS-treated animals were also morphologically similar to nodules of interstitial cells that have occasionally developed in testes transplanted into the necks of newborn mice of the A strain (15). Such a transplantation results in the establishment of an endocrine imbalance involving, among other things, an apparent disturbance of the pattern of release of hypophyseal gonadotropic hormones (14). It is not altogether impossible that administration of PMS is the equivalent of, or induces, a comparable imbalance. The occasional adrenal subcapsular hyperplasia and tumors that may also be produced by an endocrine imbalance (24) are in keeping with such an interpretation. It is possible that a comparable imbalance is established at some time during a long period of treatment with estrogen. Whether or not this is the mechanism of action of the estrogen, it is interesting and perhaps significant that a possible mediary at least partially duplicated a "carcinogen" in its end effects.

Few other data are as yet available to argue for mediation by the hypophysis of the action of injected estrogen in inducing the development of testicular tumors. Administration of luteinizing hormone (7) and interstitial cell-stimulating hormone (5) have resulted in hypertrophy of Leydig cells and in evidence of increased production of androgen. No conspicuous increase in the numbers of Leydig cells in response to these substances was reported, but the period of treatment was brief. Pregnancy urine extract (4) and a lactogenic preparation (3) have had a similar action, while purified follicle-stimulating hormone is reported not to affect Leydig cells (5, 7). The development of refractoriness to these substances when they are injected into mice (17) makes their adequate testing in this respect difficult if not impossible. Whether the PMS acted upon the interstitial cells directly or by influencing some other factor is not known.

Although 9 of 15 mice receiving PMS showed discrete nodules of Leydig cells, it is improbable that testicular tumors could be induced by this treatment in view of their absence after more than 18 months of daily treatment. More data are, of course, needed to establish this point. Whether the nodules are capable of persistence or of continued growth after discontinuation of injections of PMS was not ascertained. Investigation of this possibility would be difficult inasmuch as the presence of nodules can be detected only upon microscopic study.

There was evidence of a high level of production of androgen by many of the testicular tumors in estrogen-treated mice (9). The mice receiving PMS also evinced the production of large amounts of androgen, beginning almost at the start of treatment. The morphology of the accessory glands of reproduction (10), the large size of the kidneys and their tall glomerular epithelium (16), the large size of the salivary glands (11) seen in the PMS-treated mice may all be produced regularly by the administration of androgens.

The injected PMS, as such, was probably not specifically responsible for the fibrosarcomas that developed at the site of repeated injections in 2 of the animals. At any rate, inert substances presumably lead to a similar result (22).

## SUMMARY

Fifteen male mice of the A strain were given daily injections of 50 I.U. of pregnant mare serum for periods ranging from 30 to 550 days as one means of determining whether gonadotropic hormones mediate the changes that culminate in the development of interstitial cell tumors of the testis in estrinized mice of this strain. During the first 100 days the testes enlarged slightly, a process that was followed by a slow reduction in size. After one year's treatment normal testicular size was regained or slightly exceeded. The testes never acquired the yellow or brown color that characterizes the testes of estrogen-treated mice. The accessory glands of reproduction hypertrophied greatly, evincing the production of large amounts of androgen.

In the testes of 9 of the treated animals the interstitial cells formed nodules ranging in area from one-third to twice the cross sectional area of a tubule. The nodules were fairly well localized and occupied various positions, the most frequent being in the region of the mediastinum testis. These nodules resembled young nodules in the development of testicular tumors in estrogen-treated mice. Local invasions were not seen and lymph nodes were not involved.

In the testes of 4 animals the interstitial cells were increased in number throughout the testis, but did not form nodules. No interstitial cell change was seen in 2 animals.

## REFERENCES

1. Bonser, G. M. Malignant Tumours of the Interstitial Cells of the Testis in Strong A Mice Treated with Triphenylethylene. J. Path. & Bact., **54**:149-154. 1942.
2. Bonser, G. M., and Robson, J. M. The Effects of Prolonged Oestrogen Administration upon Male Mice of Various Strains: Development of Testicular Tumours in the Strong A Strain. J. Path. & Bact., **51**:9-22. 1940.
3. Champy, C., Wolff, R., and Coujard-Champy, C. Actions d'extraits hypophysaires du type prolactine sur le tissu interstitiel du testicule. Comp. rend. Soc. de biol., **130**:443-447. 1939.
4. Collip, J. B., Selye, H., and Thompson, D. L. Beiträge zur Kenntnis der Physiologie des Gehirnanhanges. Virchows Arch. f. path. Anat., **290**:23-46. 1933.
5. Evans, H. M., Simpson, M. E., and Pencharz, R. I. An Anterior Pituitary Gonadotropic Fraction (ICSH) Specifically Stimulating the Interstitial Tissue of Testis and

Ovary. Cold Spring Harbor Symposia on Quantitative Biology, **5**:229-238. 1937.

6. Gardner, W. U. Testicular Tumors in Mice of Several Strains Receiving Triphenylethylene. Cancer Research, **3**: 92-99. 1943.
7. Greep, R. O., Fevold, H. L., and Hisaw, F. L. Effects of Two Hypophyseal Gonadotropic Hormones on the Reproductive System of the Male Rat. Anat. Rec., **65**:261-271. 1936.
8. Hooker, C. W., Gardner, W. U., and Pfeiffer, C. A. Testicular Tumors in Mice Receiving Estrogens. J. A. M. A., **115**:443-445. 1940.
9. Hooker, C. W., and Pfeiffer, C. A. The Morphology and Development of Testicular Tumors in Mice of the A Strain Receiving Estrogens. Cancer Research, **2**:759-769. 1942.
10. Hooker, C. W., and Strong, L. C. Duplication of the Seminal Vesicles in Mice of the C Strain. Anat. Rec., **81**: 333-349. 1941.
11. Lacassagne, A. Dimorphisme sexuel de la glande sous-maxillaire chez la souris. Compt. rend. Soc. de biol., **133**:180-181. 1940.
12. Lane, C. E. Some Influences of Oestrin on the Hypophyseal-Gonad Complex of the Immature Female Rat. Am. J. Physiol., **110**:681-685. 1935.
13. Moore, C. R. Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. VI. Testicular Reactions in Experimental Cryptorchidism. Am. J. Anat., **34**:269-316. 1924.
14. Pfeiffer, C. A. The Effects of an Experimentally Induced Endocrine Imbalance in Female Mice. Anat. Rec., **75**: 465-491. 1939.
15. Pfeiffer, C. A. Interstitial Cell Stimulation and Luteinization under the Influence of Male and Female Hypophyses. Yale J. Biol. & Med., **14**:619-630. 1942.
16. Pfeiffer, C. A., Emmel, V. M., and Gardner, W. U. Renal Hypertrophy in Mice Receiving Estrogens and Androgens. Yale J. Biol. & Med., **12**:493-501. 1940.
17. Pfeiffer, C. A., and Hooker, C. W. Experiments on the Source of Ovarian Androgen in the Mouse. Anat. Rec., **83**:543-571. 1942.
18. Pfeiffer, C. A., and Hooker, C. W. Early and Late Effects of Daily Treatment with Pregnant Mare Serum upon the Ovary of Mice of the A Strain. Anat. Rec., **84**:311-329. 1942.
19. Pfeiffer, C. A., and Kirschbaum, A. Relation of Interstitial Cell Hyperplasia to Secretion of Male Hormone in the Sparrow. Anat. Rec., **85**:211-227. 1943.
20. Shimkin, M. B., Grady, H. G., and Andervont, H. B. Induction of Testicular Tumors and Other Effects of Stilbestrol-Cholesterol Pellets in Strain C Mice. J. Nat. Cancer Inst., **2**:65-80. 1941.
21. Strong, L. C. The Establishment of the "A" Strain of Inbred Mice. J. Hered., **27**:21-24. 1936.
22. Suntzeff, V., Babcock, R. S., and Loeb, L. The Development of Sarcoma in Mice Following Long Continued Injections of a Buffered Solution of Hydrochloric Acid. Am. J. Cancer, **39**:56-60. 1940.
23. Woolley, G., Fekete, E., and Little, C. C. Effect of Castration in the Dilute Brown Strain of Mice. Endocrinology, **28**:341-343. 1941.
24. Woolley, G. W., Fekete, E., and Little, C. C. Gonadectomy and Adrenal Neoplasms. Science, **97**:291. 1943.

# Attempts to Abrogate Immunity to the Brown-Pearce Carcinoma*

Otto Saphir, M.D., and Max Appel, M.D.

*(From the Department of Pathology of the Michael Reese Hospital,† Chicago, and the Burnham City Hospital, Champaign, Ill.)*

(Received for publication June 25, 1943)

The cells of the reticuloendothelial system are often regarded as barriers against the development and growth of malignant tumors. Experimental substantiation of this has been obtained by attempts to suppress the functions of these cells by so called blockade methods, and by stimulation of these cells for the purpose of increasing their functional activity. The former procedure is considered to decrease, and the latter to increase resistance to tumor growth.

Blockade of the reticuloendothelial system has been accomplished by the administration of substances that are readily taken up by these cells, such as trypan blue, India ink, etc. Foulds (3) found that the treatment of rabbits with trypan blue before inoculation of the Brown-Pearce carcinoma resulted in a definite increase in the percentage of tumors in the lungs, liver, and spleen. Ludford (6) obtained similar results with a mouse tumor.

Stern and Willheim (8) stimulated the reticuloendothelial system in rats by subcutaneous injections of carotin. The treatment was followed by the disappearance of the Flexner-Jobling rat carcinoma and the Jensen rat sarcoma.

The following experiment was designed to determine whether or not interference with reticuloendothelial function could break down acquired resistance to tumor growth. For this purpose, animals that had been rendered immune to the Brown-Pearce carcinoma were used. The Brown-Pearce carcinoma is a malignant tumor of the testicle of the rabbit, which, following intratesticular transplantation, grows and metastasizes rapidly and usually kills the animal within 4 to 6 weeks.

As has been shown on many occasions (1, 2, 7), intradermal transplantation in the rabbit produces a tumor that increases in size for 3 to 4 weeks, attains its maximum dimensions, and then regresses completely. Following regression of the skin tumor the animals are immune to subsequent transplantation of this neoplasm.

For this experiment we used 12 male, brown-gray rabbits of similar breed. The animals were immunized against the Brown-Pearce carcinoma by intracutaneous transplantation of this tumor as described. In all animals the skin tumor grew well and regressed within 3 to 5 weeks. After its regression, the animals were inoculated intratesticularly with tumor in order to verify the immunity. In none of the animals that had been previously immunized by intracutaneous transplantation was there any tumor in the testicle. In 3 normal control rabbits similarly inoculated, the tumor grew well. Thus the immunity to growth of the Brown-Pearce carcinoma was apparently well established in the 12 treated animals.

A 1 per cent solution of trypan blue in physiological saline solution was used in an attempt to block the reticuloendothelial system. Of this, 3 cc. were injected intravenously every day into 9 of the 12 immune rabbits, the remaining 3 being used as controls. The injections were started 3 days before the injection of the tumor and repeated daily for 2 weeks following the injection of the tumor. It was considered important that the administration of the trypan blue be maintained over this prolonged period because of the unusual capacity of the cells of the reticuloendothelial system to proliferate. By repeated injections we hoped to keep the reticuloendothelial system functionally as inactive as possible, realizing that complete blockade of these cells is practically impossible. After the preliminary 3 day period of treatment with trypan blue a suspension of tumor cells in saline was injected intravenously in all 12 rabbits, including the 3 that did not receive the trypan blue.

None of the 3 control animals that received the intravenous injections of tumor, but did not receive trypan blue, and in which the reticuloendothelial system was thus not interfered with, showed any tumor growth. In 7 of the 9 rabbits that had received intravenous injections with both trypan blue and tumor, growth of the tumor occurred. The distribution of the tumor nodules varied. In 3 of the 7 rabbits there

* Aided by a grant from the Woman's Board and the John D. and Fannie K. Herz Fund.

† This department is in part supported by the Michael Reese Research Foundation.

was widespread dissemination of tumor nodules in the liver, kidneys, and lung; in the remaining 4, tumor was found only in the kidneys. The appearance of the tumor was strikingly different in most instances from that of the usual Brown-Pearce carcinoma. The prominent feature was the extensive necrosis, which suggested that some factor existed in the animal that was tending to inhibit the growth of the tumor. Microscopically, the typical structure of the Brown-Pearce carcinoma was found, but the necrosis was striking, and surrounding groups of tumor cells there were many macrophages.

## DISCUSSION

The fact that the cells of the reticuloendothelial system may affect the growth of a tumor is by no means a new observation. Many investigators believe that a relationship exists between neoplastic growth and the reticuloendothelial system. The relative resistance of the spleen to the growth of tumor is probably due to the large numbers of macrophages present in this organ. As mentioned above, so called blockade of the reticuloendothelial system with various dyes and other agents (3, 6) has been found to result in a definite increase in susceptibility to tumor growth. It should be emphasized again, however, that complete blockade of the reticuloendothelial system is almost impossible.

Hoch-Ligeti (4) found that after the injection of 1,2,5,6-dibenzanthracene the titer of antibodies formed in response to the administration of foreign proteins was much reduced. She believed that this is due to the effect of 1,2,5,6-dibenzanthracene on the cells of the reticuloendothelial system. If it is accepted that the cells of the reticuloendothelial system are responsible for the production of antibodies, one may be justified in assuming that blockade of these cells in immune animals by the administration of trypan blue diminishes their capacity to produce antibodies against the growth of this tumor. Production of antibodies to the growth of the Brown-Pearce carcinoma has been shown recently by Kidd (5), who demonstrated the appearance of complement-fixing antibodies in the blood of rabbits bearing Brown-Pearce carcinoma, and also in the blood of animals that had been immunized to the growth of this tumor. Although there is no definite experimental evidence to bear this out, it seems likely that the cells of the reticuloendothelial system are responsible for the formation of antibodies, and that blockade of these cells by trypan blue suppresses their ability to form antibodies against the Brown-Pearce carcinoma, and so permits the tumor to grow in animals that had been previously immunized. However, one must consider that the phagocytic activity of the reticuloendothelial cells may also be an important factor in controlling the growth and development of a tumor.

The extensive necrosis in the tumor is interesting, for it suggests that despite the administration of trypan blue, proliferation of the cells of the reticuloendothelial system and continued production of antibodies against the growth of this tumor continued, and that this formation of antibodies was sufficient to cause some necrosis of the tumor but was not sufficient completely to suppress its growth in the immune animals treated with trypan blue as it did in immune animals that were not so treated.

In conclusion, it should be emphasized again that complete blockade of the reticuloendothelial system is impossible. This was borne out by preliminary experiments in which we had tried to break down immunity to the Brown-Pearce carcinoma by this method and in which we had been uniformly unsuccessful. The reason for our failures in these preliminary experiments apparently was due to the fact that we did not continue the administration of trypan blue after the animals had received their intravenous injections of tumor cells. Thus after the tumor cells had been injected the cells of the reticuloendothelial system continued to proliferate sufficiently to prevent growth of the tumor. In the present experiment we prevented this from occurring by continuing the injections of trypan blue for a period of 2 weeks after the transplantation of the tumor, thus trying to obtain as much suppression of the reticuloendothelial system as possible.

## SUMMARY

Prolonged intravenous administration of trypan blue in rabbits that had been rendered immune to the Brown-Pearce carcinoma by intracutaneous transplantation, resulted in some animals in abrogation of this immunity. In 7 of 9 tumor-immune rabbits so treated, tumor growth followed intravenous injection with a suspension of tumor cells.

## REFERENCES

1. Besredka, A., and Gross, L. Vaccination du lapin contre l'épithélioma sous-cutané. Compt. rend. Acad. d. sc., **202**:1217-1219. 1936.
2. Besredka, A., Magat, I., Laval, P., and Besnard, P. L'épithélioma intracutané du lapin et son pouvoir immunisant. Ann. Inst. Pasteur, **56**:125-136. 1936.
3. Foulds, L. The Effect of Vital Staining on Distribution of Brown-Pearce Rabbit Tumour. Scient. Rep. Invest. Imp. Cancer Research Fund, **10**:21-31. 1932.
4. Hoch-Ligeti, C. Studies on the Effect of Carcinogenic Hydrocarbons on Immunity Reactions. Brit. J. Exper. Path., **22**:233-240. 1941.
5. Kidd, J. G. A Distinctive Substance Associated with the

Brown-Pearce Rabbit Carcinoma. II. Properties of the Substance: Discussion. J. Exper. Med., **71**:351-371. 1940.

6. Ludford, R. J. The Influence of Vital Dyestuffs and of Metallic Colloids on Resistance to Transplantable New Growths. Scient. Rep. Invest. Imp. Cancer Research Fund, **10**:1-20. 1932.
7. Pearce, L., and Brown, W. H. Studies Based on a Malignant Tumor of the Rabbit. IV. The Results of Miscellaneous Methods of Transplantation, with a Discussion of Factors Influencing Transplantation in General. J. Exper. Med., **37**:811-828. 1923.
8. Stern, K., and Willheim, R. Beeinflussung des reticuloendothelialen Systems durch Carotin: nebst Versuchung über Einwirkung des Carotins auf das Tumorwachstum. Ztschr. f. d. ges. exper. Med., **97**:354-361. 1935.

# The Importance of Dosage in the Intradermal Immunization against Transplantable Neoplasms

Ludwik Gross, M.D.*

*(From the Institute for Medical Research, Christ Hospital, Cincinnati, Ohio)*

(Received for publication June 25, 1943)

It has been observed in recent studies (9) that mice of a pure inbred line can be immunized against a tumor that originated in an animal of the same line. The method employed for immunization (3, 4) consists of inoculating intradermally a small dose of tumor cell suspension. In certain instances the resulting intradermal tumors regress spontaneously, and animals that recover are immune to reinoculation with the same neoplasm. In many instances, however, and especially when larger doses of tumor are inoculated, the intradermal tumors do not regress, but grow progressively, and eventually kill their hosts (3, 9). The present studies, a continuation of this previous work, were designed to determine in systematic experiments whether the hitherto high mortality rate, incident to progressive growth of many of the intradermal tumors, could be substantially reduced by using more diluted tumor cell suspensions for the purpose of immunization. In addition, experiments were designed to determine the degree of immunity acquired by male and female mice that recovered from the intradermal neoplasms.

## MATERIALS AND METHODS

*Animals.*—All mice were of the C3H line (9) bred in this laboratory by brother-to-sister mating. Males and virgin females, 6 to 8 weeks old, were used for inoculations.

*Tumor.*—Sarcoma 1, used in this study, was induced 12 months ago with methylcholanthrene in a C3H male mouse (9), and has been transplanted since that time under the skin of young, adult C3H males every 7 to 10 days. The growth of this tumor was apparently constant during these passages. Tumor cell suspensions were prepared in the usual manner (9) from a 7 to 10 day old subcutaneous sarcoma. A 20 per cent suspension was first prepared, and then diluted with 0.85 per cent solution of sodium chloride to obtain tumor suspensions varying in concentration from 0.1 to 20 per cent. At the time of each experiment the cell suspension was tested for bacterial sterility; microscopic sections of the tumor were also prepared. The histological appearance of this tumor, a spindle cell sarcoma (9), remained unchanged throughout the study.

In most experiments the suspension was prepared from a single tumor, but in two experiments (Table IV) several subcutaneous tumors had to be pooled because such large quantities of tumor suspensions were needed for the numerous animals and the massive test inoculations.

*Inoculation.*—The intradermal inoculations were made with the standard volume of 0.02 cc. of tumor suspensions varying in concentration from 0.1 to 20 per cent. These inoculations were made into the freshly shaved or plucked skin in the middle of the back of the mouse, the technic used having been described elsewhere (9). The subcutaneous inoculations (Table IV) were made under the skin of the back. A drop of collodion was used to prevent immediate escape of the injected cell suspension; this precaution was necessary particularly when large quantities of tumor suspension were injected under the skin.

## RESULTS

*Influence of dose on the incidence of takes. The minimum tumor dose (MTD).*—In several successive experiments, groups of male and female mice were inoculated intradermally with a standard volume (0.02 cc.) of tumor cell suspensions varying in concentration from 0.1 to 20 per cent (Table I).

Tumors appeared within 7 days following inoculation of the 10 and 20 per cent suspensions, and within 12 days in animals inoculated with suspensions varying in concentration from 0.5 to 5 per cent. The incidence of takes was 100 per cent in all animals inoculated with tumor suspensions varying in concentration from 1.25 to 20 per cent. In most instances (except in Experiment 4) the 1 per cent suspensions produced neoplasms. Eleven mice, inoculated with a 0.5 per cent suspension, all developed tumors. None of the animals inoculated with 0.2 or 0.1 per cent suspensions de-

* Now captain, Medical Corps, United States Army.

veloped neoplasms. Accordingly, 0.02 cc. of a 0.5 per cent suspension of tumor cells prepared from a 7 to 10 day old subcutaneous sarcoma, and inoculated intradermally, was designated as the minimum tumor dose (MTD). The same minimum tumor dose was also found to be sufficient to produce a tumor when injected under the skin (Table IV).

TABLE I: INCIDENCE OF TAKES FOLLOWING INTRADERMAL INOCULATION OF 0.2 CC. OF TUMOR CELL SUSPENSIONS VARYING IN CONCENTRATION FROM 0.1 TO 20 PER CENT

| Experiment No. | Tumor, per cent concentration | Sex | No. of mice inoculated | No. of mice developing tumors | Takes, per cent |
|---|---|---|---|---|---|
| 1 | 20 | F | 10 | 10 | 100 |
| | 5 | M | 10 | 10 | 100 |
| | 5 | F | 10 | 10 | 100 |
| | 1.25 | F | 6 | 6 | 100 |
| 2 | 10 | F | 7 | 7 | 100 |
| | 5 | F | 7 | 7 | 100 |
| | 2.5 | F | 7 | 7 | 100 |
| | 1.25 | F | 7 | 7 | 100 |
| 3 | 10 | F | 5 | 5 | 100 |
| | 5 | F | 5 | 5 | 100 |
| | 1 | F | 5 | 4 | 80 |
| | 0.2 | F | 5 | 0 | 0 |
| 4 | 10 | F | 7 | 7 | 100 |
| | 1 | F | 7 | 0 | 0 |
| | 0.1 | F | 7 | 0 | 0 |
| 5 | 10 | M–c * | 5 | 5 | 100 |
| | 1 | M–c | 6 | 5 | 83 |
| | 0.1 | M–c | 6 | 0 | 0 |
| 6 | 10 | M | 10 | 10 | 100 |
| | 10 | F | 11 | 11 | 100 |
| | 1 | M | 12 | 9 | 75 |
| | 1 | F | 12 | 9 | 75 |
| 7 | 5 | M | 12 | 12 | 100 |
| | 0.5 | M | 5 | 5 | 100 |
| 8 | 5 | M | 5 | 5 | 100 |
| | 0.5 | M | 6 | 6 | 100 |
| Total | 1.25–20 | | 124 | 124 | 100 |
| | 0.5 – 1 | | 53 | 38 | 72 |
| | 0.1 → 0.2 | | 18 | 0 | 0 |

* Castrated males.

*Susceptibility of mice that did not react to the first inoculation of sarcoma.*—Thirty of the 33 animals that did not develop tumors following the first inoculation of a small dose of tumor cell suspension were reinoculated intradermally with the same sarcoma. As shown in Table II, all these animals developed tumors following reinoculation with 0.02 cc. of either a 5 or 10 per cent suspension, *i.e.,* 10 or 20 MTD, of sarcoma. Thus a preliminary inoculation with a subeffective dose of tumor, insufficient to produce a neoplasm, did not render the animals immune to reinoculation with the same sarcoma.

*The influence of dose on the incidence of spontaneous regression of the intradermal tumors.*—Tumors produced by intradermal inoculations of cell suspensions of sarcoma either grew progressively and killed their hosts in from 27 to 86 days (average 45 days) or, as has been observed in previous studies (3, 9), persisted only temporarily and eventually disappeared (Figs. 1 to 3) in from 16 to 52 days following inoculation (average 28 days).

The incidence of spontaneous regression of the intradermal tumors was substantially higher in all groups of animals that had been inoculated with small doses of sarcoma than in those injected with the more concentrated tumor cell suspensions. As shown in Table III, tumors regressed spontaneously in 25 of 38 mice (66 per cent) inoculated with tumor cell suspensions varying in concentration from 0.5 to 1.25 per cent; *i.e.,* with 1 to 2.5 MTD of sarcoma. Eighteen of 46 mice (39 per cent) recovered from tumors that resulted from inoculation of tumor cell suspensions varying in concentration from 2.5 to 5 per cent (5 to 10 MTD), and only 11 of 43 mice (26 per cent) recovered from tumors that had developed following

TABLE II: INTRADERMAL REINOCULATION OF MICE THAT DID NOT REACT TO THE FIRST INOCULATION OF SARCOMA

| Experiment No. | First inoculated tumor, per cent concentration * | Interval, days † | Second inoculated tumor, per cent concentration * | Sex | No. of mice inoculated | No. of mice developing tumors | No. of mice tumors regressed | Takes, per cent |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 16 | 10 | F | 7 | 7 | 2 | 100 |
| 2 | 0.1 | 16 | 10 | F | 7 | 7 | 1 | 100 |
| 3 | 1 | 28 | 10 | F | 1 | 1 | 0 | 100 |
| 4 | 0.2 | 28 | 10 | F | 3 | 3 | 1 | 100 |
| 5 | 1 | 18 | 10 | M–c ‡ | 1 | 1 | 0 | 100 |
| 6 | 0.1 | 18 | 10 | M–c | 5 | 5 | 1 | 100 |
| 7 | 1 | 17 | 5 | M | 3 | 3 | 2 | 100 |
| | 1 | 17 | 5 | F | 3 | 3 | 1 | 100 |
| Total | | | | | 30 | 30 | 8 | 100 |

* 0.02 cc. injected intradermally.
† Interval between the first and second inoculations.
‡ Castrated males.

FIG. 1.—Female 1063 eleven days after inoculation.

FIG. 2.—Female 1063 twenty-seven days after inoculation.

FIG. 3.—Female 1063 thirty-eight days after inoculation.
Tumor completely disappeared.

*Typical course of intradermal immunization against sarcoma.*—Female 1063 was inoculated intradermally with 0.02 cc. of a 1 per cent suspension (2 MTD) of sarcoma 1 (Figs. 1 to 3).

inoculation of 10 and 20 per cent suspensions (20 to 40 MTD).

These data suggest that the incidence of spontaneous regression of the intradermal tumors is inversely proportional to the inoculated dose, the highest incidence of regression occurring in animals that are inoculated with the smallest doses of sarcoma (1 to 2.5 MTD).

*Intradermal reinoculation of animals that recovered from tumors. The "accelerated" and the "immediate" reactions.*—Twenty-four males and 23 females that had recovered from the intradermal tumors (20 males and 23 females from experiments reported in Table II, and 4 males from similar experiments not included in this table) were reinoculated intradermally with 0.02 cc. of a 5 per cent suspension (10 MTD) of sarcoma. The interval between the first and the second intradermal inoculations varied from 30 to 45 days for different groups of mice. The second inoculation was made into the skin of the back of the mouse, 1 to 2 cm. distant from the minute scar that had resulted from the regression of the primary intradermal neoplasm. Seven of the 24 males and 7 of the 23 females developed intradermal tumors at the site of the second inoculation. In 4 instances these tumors resembled primary takes in all respects: 2 of them grew slowly but progressively, and 2 disappeared spontaneously after 25 and 28 days. In 6 animals the tumors appeared after a shortened incubation period, the height of their evolution (Fig. 4) occurring about the fourth or fifth day; in each instance these tumors disappeared spontaneously (Fig. 5) within 16 days. This has been designated an "accelerated" reaction. Finally, in 4 mice small tumors appeared within 48 hours; these disappeared completely within 6 days following reinoculation. This has been designated the "immediate" reaction.

Table III: The Influence of Dosage of the Tumor Suspension on the Incidence of Regression of the Intradermal Neoplasms

| Experiment No. | Tumor, per cent concentration * | MTD † | Sex | No. of mice with tumors | No. of mice tumors regressed | Spontaneous regression, per cent |
|---|---|---|---|---|---|---|
| 1 | 20 | 40 | F | 10 | 4 | 40 |
| | 5 | 10 | M | 10 | 4 | 40 |
| | 5 | 10 | F | 9 | 4 | 44 |
| | 1.25 | 2.5 | F | 6 | 4 | 67 |
| 2 | 10 | 20 | F | 7 | 1 | 14 |
| | 5 | 10 | F | 7 | 3 | 43 |
| | 2.5 | 5 | F | 7 | 3 | 43 |
| | 1.25 | 2.5 | F | 2 | 1 | 50 |
| 3 | 10 | 20 | F | 5 | 1 | 20 |
| | 5 | 10 | F | 4 | 2 | 50 |
| | 1 | 2 | F | 3 | 3 | 100 |
| 4 | 10 | 20 | M | 10 | 2 | 20 |
| | 1 | 2 | M | 9 | 7 | 67 |
| 5 | 10 | 20 | F | 11 | 3 | 27 |
| | 1 | 2 | F | 9 | 5 | 56 |
| 6 | 5 | 10 | M | 4 | 1 | 25 |
| | 0.5 | 1 | M | 5 | 3 | 60 |
| 7 | 5 | 10 | M | 5 | 1 | 20 |
| | 0.5 | 1 | M | 4 | 2 | 50 |
| Total—10 –20 | | 20–40 | | 43 | 11 | 26 |
| 2.5– 5 | | 5–10 | | 46 | 18 | 39 |
| 0.5– 1.25 | | 1– 2.5 | | 38 | 25 | 66 |

* 0.02 cc. injected intradermally.

† One MTD equals 0.02 cc. of a 0.5 per cent suspension of tumor cells prepared from a 7 to 10 day old sarcoma.

As controls, 3 normal males and 3 females were inoculated simultaneously with 0.02 cc. of 5 per cent tumor cell suspensions; all developed rapidly growing neoplasms. The 3 males were destroyed with large sarcomas; 2 of the females died with tumors and 1 female recovered after 25 days.

*Degree of immunity acquired by male and female mice that recovered from the intradermal tumors.*—The 24 males and 21 females that had recovered from the primary intradermal tumors and also had survived the second intradermal inoculation, were finally submitted to a subcutaneous test inoculation of sarcoma (Figs. 6 and 8). The interval between the first intradermal and the subcutaneous test inoculation of sarcoma varied from 40 to 60 days for different groups of the immunized mice. Normal control mice of the same sex were injected simultaneously by the same route, and with equal amounts of the same tumor cell suspensions (Figs. 7 and 9). As controls for potency of the tumor suspensions, 2 groups of animals were also inoculated with 0.02 cc. of a 0.5 per cent suspension, *i.e.*, with 1 MTD of sarcoma.

As shown in Table IV, none of the 5 immunized males resisted a subcutaneous test inoculation of 5,000 MTD; *i.e.*, 2.5 cc. of a 20 per cent suspension of sarcoma. Two of 5 immunized males developed tumors following subcutaneous test inoculation of 250 MTD, *i.e.*, 0.25 cc. of a 10 per cent suspension of sarcoma, and only 2 of 5 immunized males resisted the comparatively moderate dose of 25 MTD, *i.e.*, 0.25 cc. of a 1 per cent suspension of sarcoma. Nine immunized males were inoculated with 2 MTD, *i.e.*, 0.02 cc. of a 1 per cent suspension of sarcoma, and 8 proved refractory; in 1 animal a small subcutaneous nodule appeared and was excised (microscopic diagnosis: sarcoma).

Immunized females were more resistant than males to subcutaneous test inoculations of sarcoma. Thus 2 of 5 immunized females resisted the massive test inoculation of 5,000 MTD, and 16 females proved refractory to test inoculations varying from 2 to 800 MTD of sarcoma (Table IV).

*The evolution of subcutaneous tumors.*—Twenty-

FIG. 4.—Female 1063 four days after intradermal reinoculation of sarcoma.

FIG. 5.—Female 1063 sixteen days after intradermal reinoculation of sarcoma. "Secondary" tumor disappeared.

*The "accelerated" reaction in course of intradermal reimmunization against sarcoma.*—Female 1063 that had recovered from the primary intradermal tumor (Figs. 1 to 3) was reinoculated intradermally with 0.02 cc. of a 5 per cent suspension (10 MTD) of sarcoma 1. As a result of the reinoculation a small tumor appeared (Fig. 4) and promptly disappeared (Fig. 5).

The local spotty pigmentation of the skin (Fig. 5) often appeared in the C3H mice following plucking or shaving; such pigmentation persisted for a few days and then gradually disappeared.

TABLE IV: DEGREE OF IMMUNITY ACQUIRED BY MALE AND FEMALE MICE THAT RECOVERED FROM INTRADERMAL TUMORS SUBCUTANEOUS TEST INOCULATION OF SARCOMA

| Experiment No. | Dose | | | Immunized mice | | | | | Normal control mice | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tumor, per cent concentration | cc.* | MTD † | Sex | No. of mice inoculated | No. of mice developing tumors | No. of mice dead with tumors | Takes, per cent | Sex | No. of mice inoculated | No. of mice developing tumors | No. of mice dead with tumors | Takes, per cent |
| 1 | 20 | 2.5 | 5,000 | M | 5 | 5 | 5 | 100 | M | 3 | 3 | 3 | 100 |
| | 10 | 0.25 | 250 | M | 5 | 2 | 2 | 40 | M | 5 | 5 ‡ | 4 | 100 |
| | 1 | 0.25 | 25 | M | 5 | 3 | 1 | 60 | M | 4 | 4 ‡ | 2 | 100 |
| | 1 | 0.02 | 2 | M | 9 | 1 ‡ | 0 | 11 | M | 6 | 5 | 3 | 83 |
| | 0.5 | 0.02 | 1 | — | — | — | — | — | M | 5 | 5 | 2 | 100 |
| 2 | 20 | 2.5 | 5,000 | F | 5 | 3 | 3 | 60 | F | 2 | 2 | 2 | 100 |
| | 10 | 0.25 | 250 | F | 6 | 0 | 0 | 0 | F | 7 | 7 ‡ | 4 | 100 |
| | 1 | 0.25 | 25 | F | 5 | 0 | 0 | 0 | F | 6 | 6 | 0 | 100 |
| | 0.5 | 0.04 | 2 | F | 3 | 0 | 0 | 0 | F | 5 | 5 ‡ | 1 | 100 |
| | 0.5 | 0.02 | 1 | — | — | — | — | — | F | 6 | 6 | 3 | 100 |
| 3 | 20 | 0.4 | 800 | F | 2 | 0 | 0 | 0 | F | 2 | 2 | 2 | 100 |
| | | | | — | — | — | — | — | — | — | — | — | — |
| Total of all experiments | | | | M | 24 | 11 | 8 | 46 | M | 23 | 22 | 14 | 96 |
| | | | | F | 21 | 3 | 3 | 14 | F | 28 | 28 | 12 | 100 |
| Total of mice inoculated with 2 to 800 MTD | | | | M | 19 | 6 | 3 | 32 | M | 15 | 14 | 9 | 93 |
| | | | | F | 16 | 0 | 0 | 0 | F | 20 | 20 | 7 | 100 |

* Injected subcutaneously.

† One MTD equals 0.02 cc. of a 0.5 per cent suspension of tumor cells prepared from a 7 to 10 day old sarcoma.

‡ One mouse with tumor sacrificed for microscopic studies of the subcutaneous neoplasm (sarcoma).

FIG. 6.—Immunized female 1063 fourteen days after subcutaneous test inoculation of sarcoma.

FIG. 7.—Control female 1160 fourteen days after subcutaneous inoculation of sarcoma.

*Resistance of the animal that recovered from the intradermal tumor to subcutaneous test inoculation of sarcoma.*—Female mouse 1063 that had recovered from the primary and secondary intradermal tumors (Figs. 1 to 5) was subjected to a subcutaneous test inoculation of 0.25 cc. of a 10 per cent suspension (250 MTD) of sarcoma 1. Normal control female 1160 was inoculated simultaneously by the same route and with equal amount of the same tumor suspension. Immunized female 1063 proved refractory (Figs. 6 and 8), whereas control female 1160 developed a large tumor at the site of inoculation (Figs. 7 and 9).

FIG. 8.—Immunized female 1063 thirty-one days after subcutaneous test inoculation of sarcoma.

FIG. 9.—Control female 1160 thirty-one days after subcutaneous inoculation of sarcoma. This mouse died 6 days later.

three normal males and 28 females were inoculated subcutaneously as controls with equal, or smaller, doses of the same tumor cell suspensions, and all but one developed tumors (Table IV). These tumors either grew progressively and killed their hosts in from 16 to 66 days (average 36 days), or they grew only temporarily, and regressed in from 22 to 60 days following inoculation (average 34 days). Animals inoculated with doses varying from 800 to 5,000 MTD developed rapidly growing tumors measuring up to 6 cm. in their largest diameters at the time when these animals died. Of the 22 animals that had been inoculated with doses varying from 25 to 250 MTD, 10 developed progressively growing tumors and 12 had subcutaneous neoplasms that eventually regressed. Twenty-two mice were inoculated with doses varying from 1 to 2 MTD of sarcoma and all but one developed tumors within 12 days following inoculation. In 12 animals these tumors reached only 3 to 5 mm. in diameter and then gradually regressed. Nine animals died with progressively growing sarcomas.

Thus 50 of 51 normal animals inoculated subcutaneously developed tumors, and 26 died with large sarcomas. Microscopically, these subcutaneous tumors were identical with the sarcoma described in our previous study (9).

*Immunity acquired by animals that recovered from the subcutaneous tumors.*—Of the 24 normal animals that had recovered from subcutaneous tumors, 19 (9 males and 10 females) were reinoculated intradermally with 0.02 cc. of a 5 per cent suspension (10 MTD) of sarcoma. At the same time 17 control males were inoculated intradermally with equal doses of the same tumor cell suspension. Of the 19 mice that had recovered from subcutaneous tumors, 17 proved refractory to the intradermal test inoculation of sarcoma and 2 males developed small tumors; one of these tumors was excised for microscopic study (sarcoma), and the other persisted for a few days and then gradually regressed. All 17 control animals developed tumors and 12 died with large sarcomas.

*Preliminary intradermal injection of normal organs followed by inoculation of sarcoma.*—In two successive experiments 5 males and 5 females were injected intradermally with 0.05 to 0.10 cc. of a 20 per cent suspension of ground liver, spleen, and kidneys that had been excised from normal C3H males. After an interval of 10 days these animals were inoculated intradermally with 0.02 cc. of a 5 per cent suspension, *i.e.,* 10 MTD, of sarcoma. All these mice developed tumors at the site of inoculation of the neoplasm; 5 recovered and 5 died with large sarcomas.

No resistance to sarcoma was, therefore, acquired following intradermal inoculation of normal organs from an animal of the same line, under the conditions of this experiment.

## DISCUSSION

Experiments reported in this and in previous papers (3, 7, 8, 9) have shown that accurate dosage is of paramount importance in the study of tumor immunity, and that massive doses of tumor may completely obscure any differences in resistance of the inoculated animals.

These facts were not sufficiently stressed during the early work on tumor immunity (11). Few attempts, if any, were made to use quantitative procedures. In most instances animals were inoculated with small pieces of tumor, implanted by means of a trocar, and little or no attention was paid to accurate dosage.

The use of the routine trocar method for the transplantation of tumors practically excludes the possibility of exact dosage; tumor cell suspensions have been employed, therefore, in our experiments. These suspensions were prepared from 7 to 10 day old subcutaneous sarcomas. The age of these tumors also deserves emphasis, since older tumors often become partially necrotic and therefore do not represent an equal value from the point of view of dosage of the tumor cells; thus a 1 per cent suspension prepared from a 7 to 10 day old sarcoma is probably more potent than a 1 per cent suspension prepared from a 30 to 35 day old neoplasm.

Evaluation of the minimal dose of tumor capable of producing a neoplasm is probably as important in the study of tumor immunity as is the MLD in studies on immunity against various infectious diseases. In our experiments the MTD was determined by inoculating intradermally groups of uniformly susceptible animals with a standard volume (0.02 cc.) of tumor cell suspensions varying in concentration from 0.1 to 20 per cent; 0.02 cc. of the highest dilution of tumor cells still capable of producing neoplasms in a majority of the inoculated animals was designated as the minimum tumor dose (MTD).

Experiments reported in this study clearly suggest that the mortality rate incident to progressive growth of the intradermal neoplasms could be substantially reduced by using the more diluted tumor cell suspensions for intradermal immunization; the highest incidence of regression of the intradermal neoplasms occurred in mice that had been inoculated with the minimal doses of sarcoma.

It should be emphasized that the small doses of tumor inoculated intradermally for the purpose of immunization must still be capable of producing neoplasms, since animals that did not develop tumors were not immune to reinoculation with the same sarcoma (Table II), a point already stressed in previous studies (4, 7) and confirmed in present experiments. There may be various reasons for occasional failure of a small dose to produce tumors in the inoculated ani-

mals. In the first place, the intradermal inoculation might not have been properly done, a part of the tumor suspension having been injected under the skin. The subcutaneous tissue seems, however, less susceptible than the skin to very small doses of tumor (2, 5, 7). Second, the injected tumor suspension might have partially escaped immediately after inoculation. Third, the tumor suspension might have been less active than usual; and last, slight variations in natural susceptibility of the inoculated animals must also be taken into consideration in spite of the fact that all animals were of the same inbred line.

Animals that had recovered from the intradermal tumors were in most instances (70 per cent) completely resistant to intradermal reinoculation of 10 MTD of the same sarcoma. The remainder (30 per cent) of these animals could be divided into 3 groups. In most of the mice that reacted to reinoculation, small tumors appeared after a short incubation period at the site of the second inoculation; these tumors disappeared completely within 16 days following reinoculation ("accelerated" reaction). In some animals very small tumors appeared within 48 hours at the site of reinoculation; these tumors disappeared after a few days without trace ("immediate" reaction). Finally, in a few instances the secondary tumors resembled primary intradermal neoplasms in all respects. Thus they either grew temporarily, disappearing spontaneously after several weeks, or they grew progressively and killed their hosts. There seems to be a striking analogy between the course of reimmunization against tumors and the revaccination of persons who have been previously immunized against smallpox (10).

The results of the subcutaneous test inoculations of the immunized animals with doses varying from 2 to 5,000 MTD of sarcoma suggest clearly that accurate dosage is essential in experiments dealing with the study of immunity acquired against neoplasms. In addition, there seems to be a distinct influence of sex on the degree of acquired immunity, a point already stressed in previous studies (1, 8, 9) and confirmed in present experiments. Thus all females that had recovered from the intradermal tumors were completely resistant to subcutaneous test inoculations of doses varying from 2 to 800 MTD of sarcoma, and 2 of 5 females proved refractory to the subcutaneous test inoculation of 5,000 MTD of the neoplasm. Immunized males, on the other hand, were less resistant, 6 of 19 animals developing tumors following subcutaneous test inoculation of doses varying from 2 to 250 MTD, and none of the 5 immunized males resisting the subcutaneous test inoculation of 5,000 MTD of sarcoma. These experiments suggest that immunity acquired against tumors, although substantial, is limited, especially in males, and that it can be in most instances overwhelmed by massive doses of the neoplasm.

The importance of accurate dosage of the tumor cell suspension was also evident in experiments dealing with natural susceptibility and resistance of the host. Thus the incidence of spontaneous regression of tumors resulting from either intradermal or subcutaneous inoculation was substantially higher in animals that had been inoculated with small doses of sarcoma than in those injected with the larger doses of the neoplasm (Tables III and IV). Although no systematic experiments have been made thus far on the evolution of tumors resulting from subcutaneous inoculations of small doses of tumor cell suspensions, it is evident from the data on the control animals, presented in Table IV, that subcutaneous tumors were not necessarily fatal, and that an appreciable number of mice recovered from the subcutaneous neoplasms. Animals that had recovered from the subcutaneous tumors were resistant to intradermal reinoculation of the same sarcoma.

Immunity could thus be acquired by regression of either an intradermal or a subcutaneous tumor. This does not necessarily imply, however, that the intradermal and the subcutaneous routes of inoculation are equally suitable for the purpose of immunization. Subcutaneous tumors resulting from inoculation of small doses of the neoplasm may regress in a substantial number of animals, especially in females. In this respect the subcutaneous tumors do not differ in principle from those resulting from intradermal inoculation of small doses of sarcoma (8). Observations made in previous studies (3, 1, 6, 7) with various tumors and in several species of animals suggest, however, that the mortality rate is usually higher in animals inoculated subcutaneously as compared with those injected intradermally with equal doses of the same neoplasm.

Finally, no influence of preliminary injection of organs from normal animals of the same line on subsequent inoculation of sarcoma was observed under the conditions of our experiment. In this respect the present observation did not differ from those reported in previous studies (4).

In conclusion it may be stated that certain tissues, such as the skin, are capable of anchoring the implanted tumor and preventing it from growing progressively and disseminating actively throughout the body (3). This anchoring capability of the skin is, however, a matter of degree, depending upon the inoculated dose. Whereas a small or moderate dose of tumor implanted intradermally will call forth an active immunological response, an excessive dose may overwhelm the defensive capacity of the skin and prevent the host from destroying the tumor locally. Similarly,

animals that have recovered from the intradermal tumors are, with few exceptions, completely immune to reinoculation with small or moderate doses of the same neoplasm, but are in most instances unable to resist a test inoculation with an overwhelming dose of the tumor (Table IV). Immunity to tumors, not unlike immunity to many infectious diseases, means protection only under a specified set of conditions. Very small doses of tumor seem most suitable for the purpose of immunization. Small or moderate doses only may be used for immunity tests. Thus dosage seems fundamental in the study of immunity acquired against neoplasms. In addition, there seems to be a distinct influence of sex, females being more resistant than males to the implantation of sarcoma (8). Accordingly, the following suggestions seem warranted to assure a more efficient procedure for intradermal immunization against transplantable neoplasms:

1. Choose females, if possible, for immunization.
2. Determine the minimum tumor dose (MTD) of the tumor used for immunization.
3. Inject intradermally 1 or 2 MTD of the tumor suspension.
4. Animals that do not react to this inoculation may be considered new, and should be reinoculated intradermally with the same or a slightly higher dose of the same neoplasm.
5. Allow the resulting intradermal tumors to regress completely before proceeding with reinoculation.
6. Reinject intradermally all animals that have recovered from tumors with a small dose of the same neoplasm (2 to 5 MTD).
7. Allow the "secondary" tumors, if any appear, completely to regress.

The animals can then be considered immune, although it should be emphasized that the degree of immunity thus acquired is limited, especially in males.

## SUMMARY

One hundred and ninety-five mice of the C3H inbred line were inoculated intradermally with a standard volume (0.02 cc.) of tumor cell suspensions, varying in concentration from 0.1 to 20 per cent. These suspensions were prepared from a sarcoma that had been originally induced by methylcholanthrene in an animal of the same line. The minimum tumor dose (MTD) capable of producing a sarcoma in the majority of the inoculated animals was thus determined (0.02 cc. of a 0.5 per cent suspension of tumor cells).

The incidence of spontaneous regression of the intradermal tumors was inversely proportional to the inoculated dose, the highest incidence of regression occurring in animals that had been inoculated with the smallest doses of sarcoma. Thus 25 of 38 mice, *i.e.*, 66 per cent, recovered from tumors that resulted from inoculation of 1 to 2.5 MTD of sarcoma (0.02 cc. of cell suspensions varying in concentration from 0.5 to 1.25 per cent). On the other hand, only 11 of 43 mice, *i.e.*, 26 per cent, recovered from tumors that resulted from inoculation of 20 to 40 MTD of sarcoma (0.02 cc. of 10 and 20 per cent suspensions).

Of the 47 animals that had recovered from intradermal tumors 45 survived a second intradermal inoculation of sarcoma. These 45 mice were submitted to a subcutaneous test inoculation of the same tumor. None of the 5 immunized males resisted the test inoculation of 5,000 MTD, and 6 of 19 immunized males developed tumors following test inoculations of doses varying from 2 to 250 MTD. Two of 5 immunized females resisted the test inoculations of 5,000 MTD, and 16 females proved refractory to doses varying from 2 to 800 MTD. Fifty-one normal males and females of the same line were inoculated as controls with equal or smaller amounts of the same tumor suspensions, and all but one developed tumors.

These experiments suggest that accurate dosage is of paramount importance in the study of tumor immunity, and that resistance acquired against neoplasms can be overwhelmed, especially in males, with massive doses of the tumor suspensions.

*Acknowledgment.*—The author is indebted to Miss I. P. Thuss for her careful technical assistance in this work.

## REFERENCES

1. Andervont, H. B. The Use of Pure Strain Animals in Studies on Natural Resistance to Transplantable Tumors. Pub. Health Rep., **52**:1885-1895. 1937.
2. Besredka, A., and Gross, L. De la virulence du sarcome de Souris par différentes voies d'inoculation et, en particulier, par la voie intracutanée. Compt. rend. Soc. de biol., **113**:19-20. 1933.
3. Besredka, A., and Gross, L. Du rôle de la peau dans la sarcomatose de la souris. Ann. Inst. Pasteur, **55**:402-416. 1935.
4. Besredka, A., and Gross, L. De l'immunisation contre le sarcome de la souris par la voie intracutanée. Ann. Inst. Pasteur, **55**:491-500. 1935.
5. Besredka, A., and Gross, L. Du rôle de la peau proprement dite et du tissu sous-cutanée dans l'évolution des tumeurs malignes. Compt. rend. Acad. d. sc., **205**:694-696. 1937.
6. Besredka, A., and Gross, L. De la nature de l'immunité acquise vis-à-vis de l'épithélioma chez le lapin. Ann. Inst. Pasteur, **60**:5-12. 1938.
7. Besredka, A., and Gross, L. Du rôle de la peau dans le sarcome de la poule au point de vue de la réceptivité et de l'immunité. Ann. Inst. Pasteur, **60**:465-476. 1938.
8. Gross, L. The Influence of Sex of Mice on Acquired Resistance to a Transplantable Sarcoma. Cancer Research, **1**:880-882. 1941.
9. Gross, L. Intradermal Immunization of C3H Mice against a Sarcoma That Originated in an Animal of the Same Line. Cancer Research, **3**:326-333. 1943.
10. Rosenau, M. J. Preventive Medicine and Hygiene. Sixth Edition. Pp. 1481. New York: Appleton-Century. 1935.
11. Woglom, W. H. Immunity to Transplantable Tumours. Cancer Rev., **4**:129-214. 1929.

# Polytene Chromosomes in Two Mammary Carcinomas of the Human Subject

John J. Biesele, Ph.D.,* and Herbert Poyner, M.D., F.A.C.S.

*(From the Department of Zoology and Physiology, The University of Texas, Austin, and Houston, Texas)*

(Received for publication June 14, 1943)

It was pointed out by Ehrich (3) in 1936 that the generalized doubling or quadrupling in nuclear volumes observed by him in 22 cancers of man when compared with the tissues of their origin was very likely the result either of chromosomal polyploidy or of chromosomal polymery; that is, the chromosomes in the cancers had multiplied either in number or in size to give rise to the increase in nuclear volume. Ehrich could not say which of the two was true. It is the purpose of this article to demonstrate that a multiplication within the individual chromosome was probably chiefly responsible, in that the chromosomes of two cancers from the human subject can be shown to be polytene (many stranded). This condition corresponds to Ehrich's chromosomal polymery. Polytene chromosomes have already been shown to occur in various mouse cancers (2) and in an ovarian tumor of the goldfish (1).

## MATERIAL AND METHOD

Two mammary carcinomas of human beings were studied. That designated carcinoma A was from case 1, while carcinoma B was from case 2.

*Case 1.*—Mrs. E. S. C. Aged 41 years. White. Married.

*History.*—For the past 2 years the patient had noticed a mass in the left breast that had become steadily larger.

*Examination.*—There was a mass about 5×7 cm. in the upper inner quadrant, slightly adherent to the subcutaneous tissues and the skin. A few nodes were palpable along the pectoral border, but none in the axilla.

Radiographs of the chest showed no evidence of metastasis.

A clinical diagnosis of carcinoma was made and a radical mastectomy done.

At operation several small nodes were found along the outer course of the pectoralis major and several in the axilla.

Dr. A. H. Braden returned the following report:

"Left breast: The breast contained an unencapsulated tumor in the upper, inner quadrant, 6×6×4 cm. It was fibrotic and contained a grayish white scirrhous nodule 2.5 cm. across with the typical appearance of scirrhous carcinoma. The neighboring fat contained numerous hard large lymph nodes, the largest of which was 12 mm. in diameter.

"Microscopic examination of the tumor showed a very dense stroma containing atypical glands. There was chromatic hyperplasia with great variation in size and shape and numerous atypical mitotic figures. There was some lymphocytic infiltration and hyalinization of the connective tissue.

"Sections from the nodes showed that all had been converted into metastatic nodules.

*"Diagnosis.*—Scirrhous mammary carcinoma, grade 3, with extensive glandular metastases."

*Case 2.*—Mrs. J. W. B. Aged 37 years. White. Married.

*History.*—The patient had had a mass in the right breast for the past 3½ months.

*Examination.*—Clinical examination was negative except for this mass, which was in the upper outer quadrant of the right breast and about 3×5 cm. in diameter.

Radiographs of the chest showed no evidence of metastasis.

A diagnosis of probable carcinoma of the breast was made and a biopsy done, which showed an adenocarcinoma of the duct type, grade 2. The pathological examination was made by Dr. Violet Keiller of the Hermann Hospital.

Radical mastectomy followed.

The tissue used for control was granulation tissue and skin from a 3 weeks' draining abdominal wound in a woman. This is admittedly a poor control in that the organ and tissue were not the same as in the 2 cases of cancer and the patient was different. Properly, regenerating adult female breast should have been used.

The fresh tissues were fixed in Carnoy's fluid, com-

* Fellow of The International Cancer Research Foundation.

posed of 3 parts of 95 per cent alcohol and 1 part of glacial acetic acid. The next day bits were removed from the fixative and stained by the acetocarmine press method. The slides were made permanent by dehydrating in 95 per cent alcohol and mounting in diaphane.

Drawings were made of metaphase plates under oil immersion with the aid of a camera lucida. Since a metaphase chromosome has the shape of two parallel cylinders, the width of a few well placed chromosomes lying with the plane determined by the two cylinder axes parallel to the focal plane was measured in each figure under oil; the chromatid radius used for calculating average volume was then taken as one-fourth of this width. The lengths of all chromosomes on the drawings were then measured to the nearest 0.25 mm., *i.e.*, 0.1 micron, and an average length was calculated. The average chromosome volume for the metaphase was thereupon calculated as

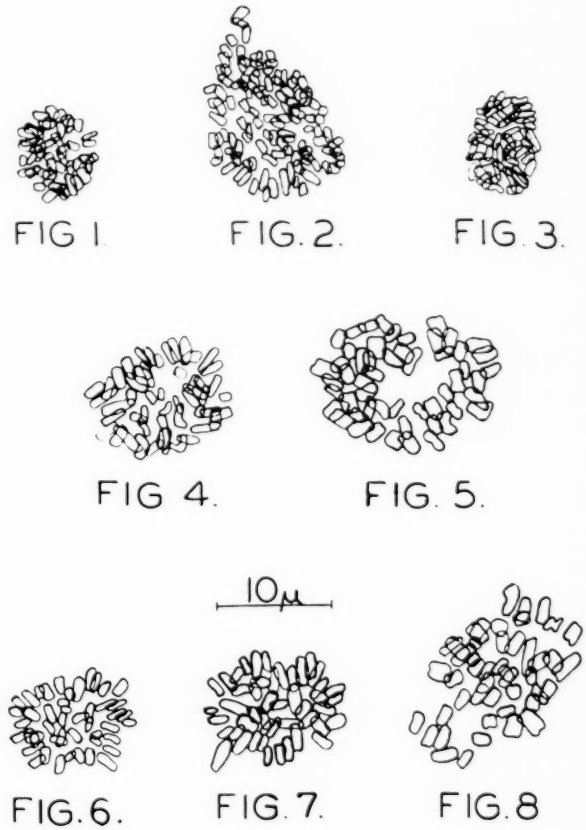
$$2 \pi r^2 \bar{h},$$

where 2 is necessary because of the two cylinders, $r$ is the chromatid radius, and $\bar{h}$ is the average length of the chromosomes. The average chromosome volume is herein given rounded off to the nearest 0.1 cubic micron. The sources of error in this whole procedure are discussed below. An average chromosome volume was calculated for each of some 25 metaphase figures in each tissue. Nuclear volume was determined as stated elsewhere (1, 2), as the average of the volumes of a short cylinder and an ellipsoid, inasmuch as the method of preparation flattened out the nuclei.

## OBSERVATIONS

Twenty-six metaphase figures of the granulation tissue had average chromosome volumes ranging from 0.3 to 0.8 cubic micron, with a mean at $0.51 \pm 0.13$ cubic micron. The distribution of the average chromosome volumes in this noncancerous tissue was unimodal, as illustrated in Fig. 9. Most of the observed chromosome numbers varied about 48, which was shown by Painter (4) to be the diploid number in man. A typical diploid mitosis is illustrated in Fig. 1. The metaphase shown in Fig. 2 was at least tetraploid, since it had about 100 chromosomes. Class I resting nuclei in the granulation tissue and skin varied from 200 to 600 cubic microns, depending on the cell type and degree of condensation of the chromatin. About 10 per cent of the nuclei were of class II in volume, and a very few nuclei, belonging to class III, had volumes 4 times the class I volume. Doubtless the tetraploid metaphase was from a class II resting nucleus. Class III nuclei were probably octoploid. In mitosis, the diploid nucleus was found to enlarge to a maximum volume in middle prophase of about 790 cubic microns, and the mitotic figure was then observed to decrease to about 525 cubic microns in prometaphase, 375 in metaphase, and 145 for each half in anaphase. A single metaphase plate from a healing skin wound on the thumb of one of us (J. B.) also proved to have chromosomes averaging 0.5 cubic micron (see Fig. 3).

In the mammary carcinoma A, 25 metaphase figures drawn at random showed average chromosome volumes ranging from 0.7 to 2.2 cubic microns. The




FIGS. 1-8

FIG. 1.—Diploid metaphase of human granulation tissue. About 48 chromosomes, average volume 0.5 cubic micron.

FIG. 2.—Tetraploid metaphase of human granulation tissue. About 116 chromosomes, average volume 0.4 cubic micron.

FIG. 3.—Diploid metaphase of healing skin of an author (J. B.). About 51 chromosomes, average volume 0.5 cubic micron.

FIG. 4.—Diploid metaphase of human mammary carcinoma A. About 48 chromosomes, average volume 0.9 cubic micron.

FIG. 5.—Diploid metaphase of carcinoma A. About 53 chromosomes, average volume 1.9 cubic microns.

FIG. 6.—Diploid metaphase of human mammary duct adenocarcinoma B. About 46 chromosomes, average volume 0.5 cubic micron.

FIG. 7.—Diploid metaphase of carcinoma B. About 47 chromosomes, average volume 1.2 cubic microns.

FIG. 8.—Diploid metaphase of carcinoma B. About 46 chromosomes, average volume 2.0 cubic microns.

figure with the smallest chromosomes may conceivably have been of a normal cell with ordinary chromosomes. Fifteen of the metaphases had diplochromosomes (see Fig. 4); the average chromosome volumes ranged from 0.8 to 1.4 cubic microns, while the mean of the 15 was $1.06 \pm 0.18$ cubic microns. This is just double the mean of the normal. Nine of the metaphases had chromosomes in the volume range 4 times the normal (see Fig. 5); they ranged from 1.5 to 2.2 cubic microns, and their mean was $1.87 \pm 0.21$ cubic microns. Fig. 9 gives the bimodal distribution of average chromosome volumes in carcinoma A. While there were some resting nuclei of class I volume, most of the resting nuclei were in class II, averaging around 900 cubic microns, and there were also a few class III resting nuclei to be seen. Corresponding to 3 classes of resting nuclei, there were 3 volume classes in middle prophase, averaging 780, 1,260, and 2,250 cubic microns for 2, 10, and 2 figures, respectively. The figure with the smallest chromosomes in metaphase was only 210 cubic microns in volume; 33 approximately diploid metaphases with diplochromosomes averaged 540 cubic microns; and 8 measurable figures with chromosomes of 4 times normal size had a mean volume of 740 cubic microns. In anaphase 14 figures, chiefly diploid with diplochromosomes, averaged 275 cubic microns. While there was a fair amount of aneuploidy in carcinoma A, all the chromosome numbers approximated the diploid. The only polyploidy was the endopolyploidy of the polytene chromosomes.

In carcinoma B, the mammary duct adenocarcinoma, the presence of three classes of nuclei and correspondingly of three classes of chromosomes was very striking. Of 25 metaphase figures drawn, 11 had average chromosome volumes in the normal size range, from 0.4 to 0.7 cubic micron, with a mean of $0.56 \pm 0.08$ cubic micron. Twelve metaphases contained diplochromosomes with calculated average volumes ranging from 0.7 to 1.4 cubic microns, the mean being $0.96 \pm 0.20$ cubic micron. Two metaphases had chromosomes of 4 times normal size; the mean of their average volumes was $1.96 \pm 0.01$ cubic microns. The three classes of chromosomes are illustrated in Figs. 6, 7, and 8, while Fig. 9 shows the distribution of average chromosome volumes. In carcinoma B, as in A, there was chromosomal aneuploidy in the diploid neighborhood, but no polyploidy. Only the building up of strand number within the chromosomes can account for the formation of different volume classes of resting nuclei. The three classes of resting nuclei were fairly distinct, as the nuclear volume distribution had three modes, which were about at 400, 800, and 1,500 cubic microns. Of 99 resting nuclei, 38 were in class I, 56 in class II, and 5 in class III. These frequencies

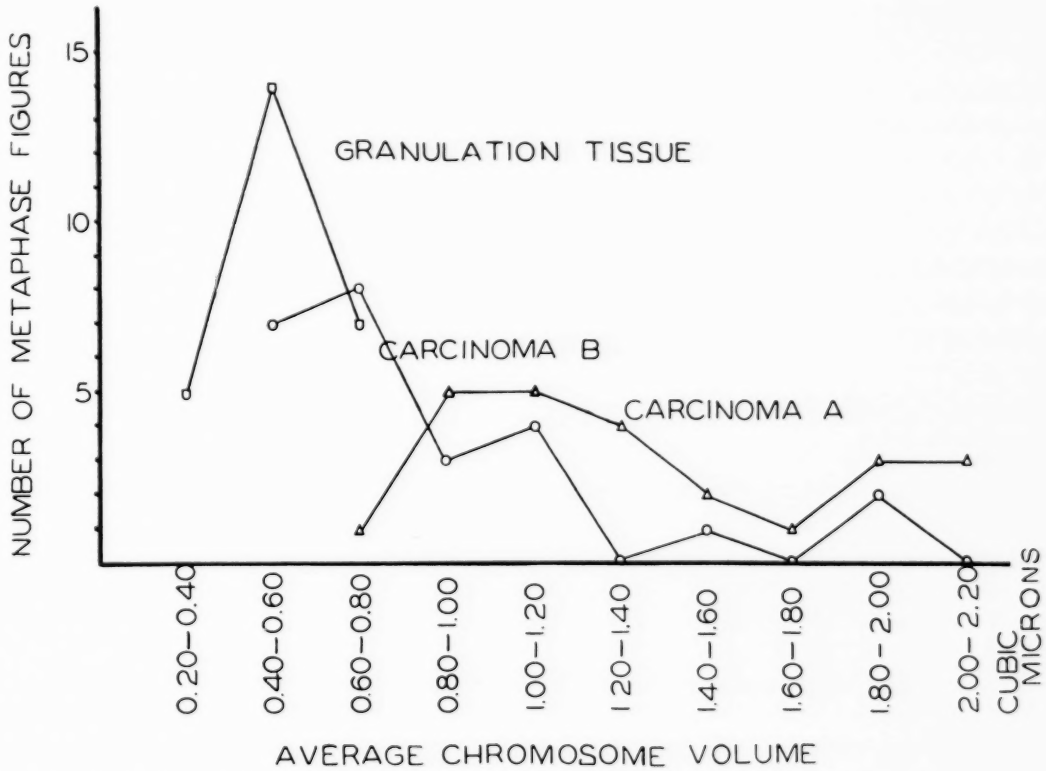



FIG. 9.—The distribution of average chromosome volumes in human granulation tissue and two mammary carcinomas. Average chromosome volume plotted against frequency.

agree fairly well with the frequencies of the several chromosome classes in metaphase. Carcinoma B was poor cytological material, and the data on mitotic phases other than the metaphase are scanty and questionable and are therefore omitted.

The human material here employed was not as suitable for the study of nucleoli as is mouse tissue. With some uncertainty it can be stated that the diploid chromosome set in normal human cells initially organizes 4 or perhaps 6 nucleoli, which often fuse. The tetraploid and octoploid chromosome sets present in nuclei of classes II and III respectively organize multiples of the basic number. In the cancers, the class II resting nuclei often seem to contain more nucleoli than do class I nuclei, and class III nuclei may contain even more. The maxima observed for the three classes in cancer were 4, 8, and 12 nucleoli.

## DISCUSSION

The sources of error in the procedure of chromosome volume determination described herein are, of course, the limited resolving power of the microscope; the observer's limitations in measuring the chromosome width in the preparation by means of camera lucida and ruler; the drawing of chromosome outlines by means of a camera lucida and a pencil line, which possesses width and often varies therein; the measurement of length of chromosome on the drawing, especially of those chromosomes that are not straight rods and of those that have not been forced to lie parallel to the focal plane by the pressure exerted in preparation; the chance that extreme pressure in preparation will flatten out the chromosomes and give them an exaggerated width; occasional elongation of chromosomes caused by pressure exerted laterally on the cover slip; the obscuring of one chromosome by another because of overlapping or clumping; the occasional measurement of a single chromosome's two chromatids as two separate chromosomes; and aneuploidy, which necessarily changes the frequency of certain lengths of chromosomes. Some of the mechanical sources of error may be avoided by studying only those portions of the slides on which the pressure was not so great as to cause rupture of the nuclei and the tissue was not rolled by a slipping cover glass. It is also advisable without doubt to use a constant lighting and light filter system throughout the observations. Notwithstanding all these possible sources of error, it hardly seems likely that their random coming into play should give polymodal volume distributions, with peaks at exact doublings and quadruplings. Rather, one should expect a unimodal curve if variation is caused solely by random errors.

The two human mammary carcinomas examined contained three classes of chromosomes: one with an average volume similar to that of chromosomes in a normal tissue, and two classes of chromosomes 2 and 4 times as large as normal chromosomes. Fig. 9 shows very well the polymodal distribution of average chromosome volumes in the cancers, as contrasted with the unimodal distribution for the granulation tissue. It should be noted that the curve for carcinoma B can be simulated in general by the addition of the curve for the normal to that for carcinoma A. Adenocarcinoma B was of lower malignancy than carcinoma A, and B had far fewer mitoses as well as a greater frequency of small nuclei. In these two cases the higher relative frequency of enlarged chromosomes was associated with the higher grade of malignancy.

The enlarged chromosomes were probably made up of a doubled or quadrupled number of strands, inasmuch as the larger resting nuclei in the cancers had greater maximal numbers of nucleoli, which are known to be organized on definite chromosomal loci. There is complete certainty on this point of an increase in the number of nucleoli with an increase in chromosome size in certain mouse cancers in which the nucleoli are plainly evident in acetocarmine preparations (2). Because of their multiple strands, the chromosomes in these two human cancers are considered to be endopolyploid, but they are not polyploid in the strict sense of duplication and complete separation of sister chromosomes within the single nucleus.

We are still uncertain whether some of the small nuclei with chromosomes of normal size in the cancers are to be thought of as nuclei of malignant cells proper, or whether they are all of supporting stromal tissue. It should be pointed out that the contentions of various authors, as reviewed elsewhere (2), that some few carcinomas do not have nuclei larger than those of the tissue of origin, does not necessarily mean that those carcinomas do not have diplochromosomes, because there is extensive polyploidy in adult vertebrate tissues, and a tetraploid nucleus with normal chromosomes has about the same volume as a diploid cancerous nucleus with diplochromosomes. Presumably a cancer could originate in one of the occasional diploid cells in a tissue most of whose cells were tetraploid.

This demonstration of polytene chromosomes in two cancers of man now makes more probable the inference drawn (2) concerning the presence of polytene chromosomes in various cancers of human beings from the many reports in the literature of greater nuclear size, more nucleoli, split chromosomes, and false meiotic divisions in cancers. While two cases are hardly sufficient to answer Ehrich's question (3) conclusively, it would seem by recourse to known polyteny in ten mouse cancers (2) and a goldfish tumor (1) that the

immediate cause of a general shift in nuclear volumes in cancerous tissues is more likely to be found in chromosomal polyteny than in polyploidy. This conclusion is likewise supported by some work now in preparation on the rat.

## SUMMARY

Two mammary carcinomas of the human subject are shown to contain chromosomes of 2 and 4 times the volume of chromosomes in human granulation tissue and in a healing skin wound, as well as chromosomes indistinguishable from those of normal tissues. There were three classes of nuclei, according to volume, in the cancers, presumably containing the three classes of chromosomes. The different nuclear classes in normal tissues can be explained on the basis of polyploidy, but no polyploidy was found in the cancers, although there was considerable aneuploidy ranging about the diploid number. Since there was an increase in the maximum nucleolar number with increase in size of resting nucleus in the cancers, the enlarged chromosomes must have been many stranded. There was a higher frequency of polytene chromosomes in the carcinoma with the higher grade of malignancy.

## REFERENCES

1. Biesele, J. J. Diplochromosomes in a Goldfish Tumor. Cancer Research, **3**:411-412. 1943.
2. Biesele, J. J., Poyner, H., and Painter, T. S. Nuclear Phenomena in Mouse Cancers. University of Texas Publication No. 4243. Austin, 1942. Pp. 68.
3. Ehrich, W. E. Nuclear Sizes in Growth Disturbances. With Special Reference to the Tumor Cell Nucleus. Am. J. M. Sc., **192**:772-790. 1936.
4. Painter, T. S. Studies in Mammalian Spermatogenesis. II. The Spermatogenesis of Man. J. Exper. Zoöl., **37**:291-338. 1923.

# Abstracts

## Experimental Research, Animal Tumors

**Experimental Brain Tumors. III. Tumors Produced with Dibenzanthracene.** ARNOLD, H., and ZIMMERMAN, H. M. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **3**:682-685. 1943.

Intracerebral implantation of pellets of 1,2,5,6-dibenzanthracene in 21 female C3H mice yielded 13 tumors. Of these, only 2 were gliomas—a glioblastoma multiforme and an ependymoblastoma. There were only 2 other intracranial neoplasms; one was a meningeal sarcoma and the other a fibrosarcoma. Eight tumors were extracranial fibrosarcomas. The remaining new growth was a squamous cell carcinoma of the scalp. Eight animals failed to develop any type of tumor. With the single exception of the possible glioma produced by Weil with a lard solution of dibenzanthracene in a white rat, the 2 gliomas reported here are the first gliogenous neoplasms produced with this chemical carcinogen.—Author's abstract.

**Single and Multiple Lung Tumours.** CAMPBELL, J. A. [London, England] *Brit. M. J.,* **2**:21-22. 1943.

In reply to inquiries, the author states that in 17 dusting experiments (*Brit. M. J.,* **1**:179-183. 1943; see abstract, *Cancer Research,* **3**:486. 1943) "the average number of lung tumours per mouse with a tumour was 2.4 for the dusted mice, compared with 1.9 for the controls." The largest number of tumors in any one mouse was 17 for the dusted group and 9 for the controls.—E. L. K.

**On the Quantitative Evaluation of Experimental Skin Carcinogenesis by Methylcholanthrene. The Factors of Dosage, Time, Spacing of Applications, and the Multiplicity of the Carcinogenic Response.** CRAMER, W., and STOWELL, R. E. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research,* **3**:668-681. 1943.

Further evidence is adduced to show that a continuous exposure of the mouse skin to methylcholanthrene, which involves the application of relatively large total doses in order to induce skin cancer, is not essential. Skin cancer can be induced by much smaller doses if the applications are made at long intervals such as 1 month. This implies a discontinuous exposure of the skin to the carcinogen.

These facts necessitate revising current conceptions of the mode of action of chemical carcinogens. In discussing these implications the following conclusions have been drawn. Increasing the dosage applied to a large area of skin increases the number of multiple skin cancers. Minimal dosages induce single skin cancers even in the more susceptible individuals of a strain. A multiplicity of skin cancers is therefore not in itself an indication of an increased susceptibility of the skin to a carcinogen.

Increasing the dosage shortens the time at which cancer develops in resistant animals; it does not materially shorten the time of induction in the most susceptible animals. The quantitative evaluation of the carcinogenic potency of chemical substances should be based on the use of minimal doses of the carcinogen. It can be determined by the percentage of cancerous tumors and the dose, rather than by the percentage of cancerous animals and the time of induction.

The etiology of multiple skin cancer in man is discussed in the light of our findings.—Author's abstract.

**Leberextrakte von Primärcarcinomen bewirken Krebs bei der Maus. [Induction of Cancer in the Mouse by Extracts of Liver Carcinoma.]** CRACIUM, E. C., ZUGRÁVESCU, I., URSU, A., and MANOLESCU-MOTOC, F. *Chem. Zentralbl.,* **112**:783. 1941. *Bull. Sect. sc. Acad. roum.,* **23**:36-47. 1940.

The liver from a person who had died of primary liver carcinoma was minced and extracted with benzene. At the boundary between the benzene and water phases, a fat-like material separated from which a waxy material was obtained by distillation. From the benzene fraction a solid material was prepared. The tissue residue was dried, powdered, and extracted in succession with benzene, cold ether, and warm ether. From the warm ether extract a more solid material and an oil were obtained. The solid material was extracted with cyclohexane. The different extracts were injected subcutaneously into mice twice weekly. With the cold ether extract, it was possible to produce tumors in mice near the site of injection. The tumors formed pulmonary metastases, but were not transplantable.—Of the hydrocarbons prepared by Diels, only the 3′-methylcyclopentenophenanthrene was found to be carcinogenic. (Translation of abstract in Chem. Zentralbl.).—C. J. L.

**The Metabolism of 1,2-Benzanthracene in Mice and Rats.** BERENBLUM, I., and SCHOENTAL, R. [Univ. of Oxford, Oxford, England] **With an Appendix on Absorption Spectra.** HOLIDAY, E. R. [London Hosp., London, England] *Cancer Research,* **3**:686-690. 1943.

A fluorescent phenolic derivative has been isolated from the feces of mice and rats injected with 1,2-benzanthracene by the intraperitoneal route. On methylation of the metabolite a product was obtained that possessed identical chromatographic behavior, fluorescence spectrum, and absorption spectrum (in the range longer than 300 mμ) with those of synthetic 4′-methoxy-1,2-benzanthracene. It is suggested, therefore, that the metabolite is 4′-hydroxy-1,2-benzanthracene. The mechanism of metabolic oxidation of carcinogenic hydrocarbons is discussed briefly.—Authors' summary.

---

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

**Espectro de fluorescencia del colesterol irradiado con Cd. y del 1-2 benzopirene. [Fluorescence Spectrum of Cd.-Irradiated Cholesterol and of 1,2-Benzpyrene.]** ROFFO, A. E., JR. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer,* **18**:949-959. 1941.

The author studied the fluorescence spectra obtained from 1,2-benzpyrene, from natural purified cholesterol, and from Cd.-irradiated cholesterol [irradiated with a cadmium vapor lamp] in a benzol solution of 1:20,000.

The fluorescence spectrum of benzpyrene extends from 3,950 to 4,900 Å. It shows bands with maxima at 4,140, 4,260, and 4,460 Å, and minima at 4,180 and 4,425 Å. The spectrum of Cd.-irradiated cholesterol lies between 3,800 and 5,000 Å. It also shows 3 bands, the maxima being at 4,050, 4,280, and 4,460 Å, and minima at 4,160 and 4,425 Å. The total fluorescence of the Cd.-irradiated cholesterol is 0.8 that of the 1,2-benzpyrene. The natural purified cholesterol does not show any fluorescence in the concentration used.—M. D.-R.

**The Use of Radioactive Tracers in Biology and Medicine.** HAMILTON, J. G. [Crocker Radiation Lab., Univ. of California, Berkeley, Calif.] *Radiology,* **39**:541-572. 1942.

The author gives a general discussion concerning the type of investigation in which radioactive tracers are useful and presents data on the distribution of radioactive phosphorus in normal and tumor-bearing human beings and laboratory animals. Some of the studies of iodine metabolism in the normal, diseased, and tumor-bearing thyroid gland are reviewed as well as the work done on the metabolism of radioactive calcium, strontium, carbon, nitrogen, and iron, in plants and animals. The use of radioactive elements in the investigation and treatment of tumors is only one aspect of this general review.—C. E. D.

**Spontaneous Primary Hepatomas in Mice of Strain C3H. II. The Influence of Breeding on Their Incidence.** BURNS, E. L., and SCHENKEN, J. R. [Louisiana State Univ. Sch. of Med., New Orleans, La.] *Cancer Research,* **3**:691-692. 1943.

The incidence of hepatomas was observed in 133 untreated breeding and nonbreeding strain C3H mice over 12 months of age. Although the number of animals in some groups was rather small, the results of these studies suggested that there was a relatively equal incidence of liver tumors in the nonbreeding animals of both sexes, 6% in the males and 10% in the females. In breeding animals the incidence of hepatomas was 27% in the males and 0% in the females. There was no great difference in the average age at which the neoplasms appeared in the various groups.—Authors' abstract.

**Spontaneous Primary Hepatomas in Mice of Strain C3H. III. The Effect of Estrogens and Testosterone Propionate on Their Incidence.** SCHENKEN, J. R., and BURNS, E. L. [Louisiana State Univ. Sch. of Med., New Orleans, La.] *Cancer Research,* **3**:693-696. 1943.

Because the incidence of spontaneous liver tumors is higher in male than in female mice of strain C3H, the influence of the male sex hormone and of estrogens on the tumor rate was studied. Nonbreeding mice were used in the experiment. Forty-eight were treated with testosterone propionate, 69 with α-estradiol benzoate, and 39 with ketohydroxyestrin. None of the 24 males and 4% of the 24 females injected with testosterone propionate developed hepatomas; 25% of the 59 males and none of the 10 females injected with α-estradiol benzoate developed hepatomas; 17% of the 25 males and none of the 9 females injected with ketohydroxyestrin exhibited hepatomas.—Authors' abstract.

**Spontaneous Primary Hepatomas in Mice of Strain C3H. IV. A Study of Intracytoplasmic Inclusion Bodies and Mitochondria.** BURNS, E. L., and SCHENKEN, J. R. [Louisiana State Univ. Sch. of Med., New Orleans, La.] *Cancer Research,* **3**:697-701. 1943.

Intracytoplasmic inclusion bodies and mitochondria were studied in the liver and hepatoma cells of 88 strain C3H mice. Two types of intracytoplasmic inclusion bodies were found. One was a large hyaline body found almost exclusively in the cytoplasm of tumor cells; the other was a smaller lipoprotein body found exclusively in the cytoplasm of the tumor cells. The staining reactions of these bodies are described and their possible modes of origin discussed. Mitochondrial studies made under conditions that did not permit accurate assay of their number or forms suggested that there were few mitochondria in hepatoma cells as compared to nonneoplastic cells. It is also suggested that the administration of testosterone propionate did not alter the number, and that the administration of α-estradiol benzoate reduced the number, of mitochondria in nonneoplastic liver cells.—Authors' abstract.

**Tumor Inhibitor Studies. II. The Effect of Plant Hormones on Tumor Growth.** KLINE, B. E., and RUSCH, H. P. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Cancer Research,* **3**:702-705. 1943.

Twenty-three plant hormones were tested for their effect upon the takes and subsequent growth of the Flexner-Jobling rat carcinoma, the Walker rat carcinosarcoma 256, and on a transplantable fibrosarcoma of the mouse. The following results were obtained: (1) When tumor mince was allowed to stand before inoculation in direct contact with the plant hormones a pronounced decrease in the number of takes and subsequent growth rate was generally observed. (2) Subcutaneous injection of the same substances, however, exerted no significant effect upon neoplasms already established.—Authors' abstract.

**Observations on a Chemically Induced Chicken Tumor Containing an Antigen Related to That of a Leukosis Sarcoma Agent.** GOTTSCHALK, R. G. [Cornell Univ. Med. Coll., New York, N. Y.] *Cancer Research,* **3**:649-667. 1943.

The problem of the relationship between filterable and nonfilterable chicken tumors was reinvestigated. A nonfilterable chicken tumor (sarcoma 16) originally induced by methylcholanthrene was transmitted for 27 generations in chickens. In young chicks the tumor was invasive and often metastasized, but in older birds it frequently regressed. The frequency of regression was in direct relation to the age of the chickens at time of injection.

No erythroleukosis or myeloleukosis was found in any of the chickens injected with sarcoma cells, 190 of which

were observed for more than 2 months. All attempts to isolate a filterable agent from the tumor failed. Sarcoma 16 grew readily in newborn ducklings, but no filterable agent was found in the duck-grown tumor.

The regression of sarcoma 16 rendered chickens resistant to a subsequent injection of a filterable agent of leukosis and sarcoma (agent 13).

The resistance of chickens to the growth of sarcoma 16 was proportional to the amount of antibodies to agent 13 in their sera. Chickens with sarcoma 16 that had regressed had neutralizing antibodies to agent 13 in much larger amounts than normal birds of the same age. The sera of chickens in which sarcoma 16 was growing but did not metastasize also strongly neutralized agent 13. The sera of chickens having tumors and metastases contained no neutralizing antibodies. Sarcoma 16 appeared or recurred in a few chickens several months after the graft of particles; these birds had a smaller amount of neutralizing antibodies to agent 13 than chickens in which sarcoma 16 did not recur.

Neutralizing antibodies to agent 13 were also produced by the injection into rabbits of high speed sediments from sarcoma 16. These antibodies could not be absorbed by cells from normal chicken spleen, which removed the complement-fixing antibodies. The injection into rabbits of heavy material from normal chicken spleen did not produce neutralizing antibodies to agent 13.

It is concluded that a nonfilterable chemically induced chicken tumor (sarcoma 16) contains an antigen not found in normal fowl cells. This antigen is related to an antigen contained in a filterable agent of leukosis and sarcoma (agent 13).

It is possible that the specific antigen is contained in a tumor agent that loses its infectivity when freed from cells, or that the nature of the association of the specific antigen with tumor cells precludes the isolation from the cells of an active tumor agent.—Author's abstract.

**Oral Papillomatosis of Rabbits: A Virus Disease.** Parsons, R. J., and Kidd, J. G. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **77**:233-250. 1943.

It was found that small benign papillomas occur quite frequently on the oral mucous membranes of domestic rabbits. These papillomas contain a filterable virus that is very stable. The virus was not inactivated at −22° C. and retained its activity when kept at 65° C. for 30 minutes. The virus was also found in the mouths of rabbits having no growths. It is believed that the virus may be spread by transfer from the mother to the young during the period of suckling and that it may be latent in the mouth, doing no harm unless the mucous membrane is injured. The virus has a special affinity for the mucous membranes and differs from the Shope virus.—D. S.

**A Pure Strain of Rous Sarcoma Cells.** Tenenbaum, E. [Hebrew Univ., Jerusalem, Palestine] *Brit. J. Exper. Path.*, **24**:56-58. 1943.

Rous sarcoma cells have been cultivated *in vitro*, without the addition of normal tissue, for a period of 6 months. The character of the cultures depended upon the amount of embryonic extract in the culture medium. With little or no embryonic extract liquefaction of the culture medium did not occur, and the spindle cells that migrated from explants formed continuous sheet growths.

It was not possible to maintain growth for more than 6 months (40 to 50 passages). After the first 30 passages growth declined. The cells underwent pronounced alterations, degenerated, and died. During the entire period of cultivation the cells retained their malignant properties (tested by inoculating cultures into chicks).—R. J. L.

**Studies on Rous Sarcoma Cells Cultivated in Vitro. II. Morphologic Properties of Rous Sarcoma Cells.** Tenenbaum, E., and Doljanski, L. [Hebrew Univ., Jerusalem, Palestine] *Cancer Research*, **3**:585-603. 1943.

The alterations undergone by cells of the Rous sarcoma growing *in vitro* are described and illustrated. There is a pronounced hypertrophy of the individual cells that may proceed to the formation of cells of monstrous dimensions. These retain, however, the form and architecture of their prototypes, the basophilic round cell and the spindle cell. The cultures also contain elements of unusually small sizes. Next to cell hypertrophy, changes in the nucleoplasm are conspicuous. In many cells, granular eosinophilic material accumulates in the center, leaving a clear zone between it and the nuclear membrane. Clearly defined, small, rounded, homogeneous intranuclear inclusions that stain bluish gray with Giemsa's method are often found. Similar larger bodies occur singly or may fill the nucleus. The nucleoli are frequently hypertrophic and may become broken into fragments. The entire nucleus becomes vesicular and almost free from basophilic chromatin. It is often extremely lobulated. The most striking of the cytoplasmic alterations is a marking off of the central zone with the formation of a roundish, more deeply staining area at the center of the cell. The peripheral cytoplasmic layer often contains basophilic thread-like and granular structures that sometimes form extensive nets, and in addition, eosinophilic material in the form of fine granules and clumps. In a few of the cells crystalline structures also are encountered. Vacuoles are present in abundance. The large round cells may project pseudopodia in the form of rigid rods that move freely as if on a jointed base. The distal ends may swell to spherical masses that may become detached. Atypical mitosis is frequent. Multinucleated cells are often found.

The nature and significance of these changes are discussed.—C. J. L.

**Squamous Cell Carcinoma of the Bovine Horn.** Frank, E. R., and Roderick, L. M. [Kansas State Coll., Manhattan, Kans.] *J. Am. Vet. M. A.*, **99**:131-132. 1941.

This tumor is rarely reported in this country but has been noted in India. The growth described herein followed injury and removal of the horn. The tumor was removed but recurred 5 months later.—E. E. S.

**Preliminary Note on Hypertrophic Pulmonary Osteoarthropathy Associated with a Bronchogenic Giant-Cell Tumor in the Left Lung.** Poley, P. P., and Taylor, J. S. *J. Am. Vet. M. A.*, **99**:144. 1941.

A large mass in the lung of a dog was visible on roentgenographic examination and was associated with cough and bony symmetrical enlargements of the 4 legs.—E. E. S.

# Clinical and Pathological Reports

**Malignancy.** Chance, H. C. [Pineville, Ky.] *Kentucky M. J.*, **39**:397-400. 1941.

The author presents his viewpoint on the symptoms, diagnosis, and treatment of various types of malignant neoplastic disease.—J. L. M.

## Heredity

**Glioma of the Retina in Successive Generations.** Benedict, W. L., and Parkhill, E. M. [Mayo Clinic, Rochester, Minn.] *Am. J. Ophth.*, **26**:511-521. 1943.

Four cases are reported comprising 2 instances of the occurrence of retinal glioma in successive generations.—E. C. R.

**Familial Incidence of Retinoblastoma. With Genealogic Chart.** Falls, H. F. [Univ. of Michigan, Ann Arbor, Mich.] *Am. J. Ophth.*, **25**:42-47. 1942.

Observations are presented on a family in which 6 members of a group of 10 siblings showed the disease. A genealogic chart is included.—E. C. R.

## Diagnosis—General

**Early Symptoms of Malignant Tumors Apparent in the Handwriting. Editorial.** Kanfer, A. *Rev. Gastroenterol.*, **10**:183. 1943.

A number of particular characteristics were discovered that were present in the handwriting of an overwhelming percentage of persons suffering from malignant tumors but absent in the handwriting of persons not suffering from malignant neoplastic disease.—M. E. H.

## Therapy—General

**The Administration of Egg White and Avidin Concentrates to Patients with Cancer.** Rhoads, C. P., and Abels, J. C. [Memorial Hosp., New York, N. Y.] *J. A. M. A.*, **121**:1261-1263. 1943.

Two patients, one with mammary cancer, the other with lymphatic leukemia, were fed for 30 weeks from 16 to 40 times the amount of avidin necessary to bind *in vitro* the amount of biotin in their diets. No evidence of biotin deficiency in the patients and no deviation from the expected clinical course were noted.—G. H. H.

**Preparation for Operation and Postoperative Care of the Patient with Cancer. Review of the Contemporary Advances and Analysis of Experiences with Current Clinical Procedures.** Robillard, G. L., and Shapiro, A. L. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Arch. Surg.*, **44**:840-880. 1942.

Patients with carcinoma, being chronically ill, present more than the usual group of problems in preoperative care. The factors of age, physical condition, and associated diseases, such as cardiovascular disorders, poor renal excretion, impaired liver function, diabetes, prostatism, obesity, anemia, hypertension, and pulmonary disease, are important in preparation for operation, in the operation itself, and in postoperative convalescence. Thus, anemia may contribute to the occurrence of shock, poor wound healing, tissue anoxia, and edema. Electrolyte balance should be carefully studied and maintained throughout the preoperative period and during the period of convalescence. Malnutrition and loss of weight are often found accompanied by acidosis, avitaminosis, and hypoproteinemia. Only after careful observation, investigation, and treatment of each patient with carcinoma, should operation be attempted. Careful operative procedures, knowledge of complications and their treatment, and careful management of the postoperative period are imperative if there is to be a minimal amount of danger to the patient. Consideration of these factors is presented in detail.—R. C. R.

## Radiation—Diagnosis and Therapy

**Cancer of the Bladder and Endoscopic Use of Radium.** Ajamil, L. F., and Portilla, R. [Cancer Inst., Havana, Cuba] *Urol. & Cutan. Rev.*, **46**:11-13. 1942.

The authors present 5 cases of apparent cure of bladder carcinoma by radiation therapy. The patients were followed from 2 to 7 years.—V. F. M.

**The Complications of Irradiation Treatment of Carcinoma of the Cervix.** Cosbie, W. G. [Univ. of Toronto, and Toronto Gen. Hosp., Toronto, Canada] *Am. J. Obst. & Gynec.*, **42**:1003-1008. 1941.

The author concludes that if a lethal irradiation dose is to be delivered to carcinoma either in the primary site or extending out widely into the pelvis, there is always the danger of irradiation beyond the tolerance of adjacent normal tissue. It is felt that certain complications must be accepted as the risk of treatment in advanced cases that are beyond the scope of any other form of therapy. An appreciation of the complications and the resulting failures should act as a guide toward an ideal method of irradiation treatment.—A. K.

**Irradiation Therapy of Skin Cancers.** Greenberger, E. D. [McAlister, Okla.] *J. Oklahoma M. A.*, **34**:235-237. 1941.

Small lesions are treated with as much as 6,000 r at one time. This intensive therapy is not advised for larger lesions as the danger of recurrence or local dissemination is greater. Occasionally deep x-ray irradiation is supplemented by interstitial or contact radium therapy, or by massive low voltage irradiation, or by combining irradiation methods with surgery.—E. E. S.

**Advantages of Direct X-Ray Therapy.** Love, J. [Louisville, Ky.] *Kentucky M. J.*, **40**:238-242. 1942.

The author favors the use of low voltage therapy. The peculiar advantages of x-ray irradiation can be best utilized if the therapy is applied directly to the tumor. The procedure is safe, simple, and time-saving for the patient. The efficiency is good since cancerocidal doses are delivered into closely confined areas. Spectacular results and unusual cures are not claimed for the use of this newer method of therapy.—J. L. M.

**Further Experience with Pervaginal X-Radiation in the Management of Carcinoma of the Cervix.** McAlister, L. S. [Muskogee, Okla.] *J. Oklahoma M. A.*, **34**:239-241. 1941.

If the malignant growth is limited to the cervix and the cervical canal is open and easily accessible, either roentgen ray therapy or radium is advised. The author believes that

if the cervical canal is not visible, accessible, and patulous, and the growth is not limited to the cervix, then pervaginal roentgen ray therapy is less dangerous and perhaps more effective. This is equally true when the cervix is destroyed.—E. E. S.

**Roentgen Therapy of Brain and Spinal Cord Tumors in Children.** Mandeville, F. B. [Richmond, Va.] *Virginia M. Monthly*, **70**:86-90. 1943.

In a series of 136 cases of tumors of the brain and spinal cord 42.6% occurred in children. Serious attempts were made to treat tumors known to be radioresistant that had been incompletely removed surgically. The medulloblastoma was the tumor most favorably influenced by irradiation. Glioblastoma multiforme and pontine tumors were completely resistant.—M. E. H.

**Radiation Therapy of Carcinoma of the Skin.** Peterson, V. L. [Charleston, W. Va.] *West Virginia M. J.*, **37**:204-205. 1941.

It is thought advisable to treat each lesion as a squamous cell carcinoma, since many so called basal cell lesions have areas of squamous formation. The details of roentgen ray therapy are given, and precautions against the development of infection are stressed.—E. E. S.

**X-Ray Diagnosis of the Primary Malignant Tumors of the Small Intestine.** Swenson, P. C. [New York, N. Y.] *Rev. Gastroenterol.*, **10**:77-91. 1943.

A report of 14 cases with x-ray studies. The x-ray examination remains the one means by which early diagnosis can be reached.—M. E. H.

## Skin and Subcutaneous Tissues

**Carcinoma Digiti Pedi Superimposed upon Ringworm.** Berlin, C. [Municipal Hosp. Hadassah, Tel Aviv, Palestine] *Urol. & Cutan. Rev.*, **45**:320-321. 1941.

A carcinoma in the fourth interdigital space of the foot associated with ringworm of both feet is described.—V. F. M.

**An Unusual Case of Basal Cell Epithelioma.** Butterworth, T. [Reading, Pa.] *Urol. & Cutan. Rev.*, **45**:198-199. 1941.

A pedunculated lipoma-like epithelioma of the nose was successfully excised, and x-radiation administered to the site. There was no recurrence in over 3 years.—V. F. M.

**Papilloma about the Anal Region.** Drueck, C. J. [Chicago, Ill.] *Urol. & Cutan. Rev.*, **45**:581-582. 1941.

This is a brief description of perianal warts or condylomas. Cleanliness, excision, and fulguration are advised in treatment. These lesions are potential foci of malignancy, especially in the aged.—V. F. M.

**Malignant Melanoma of the Nose.** Kaplan, I. I. [New York Univ. Med. Coll., New York, N. Y.] *Arch. Otolaryng.*, **35**:85-90. 1942.

Melanosarcoma of the nose is a rare condition. The author briefly reviews the literature and adds the case reports of 2 patients. Both patients were treated by surgery and irradiation.—M. E. H.

## Nervous System

**Von Recklinghausen's Disease and Auditory Neurinomata.** Hadley, H. G. [Washington, D. C.] *Urol. & Cutan. Rev.*, **46**:122-123. 1942.

This is a concise description of the disease, with 2 case reports.—V. F. M.

**Neurofibroma (Neurinoma) of the Foot.** Hauser, E. D. W. [Chicago, Ill.] *J. A. M. A.*, **121**:1217-1219. 1943.

Report of a case. The tumor apparently arose from the deep plantar nerve.—G. H. H.

**Presacral Sympathicoblastoma in an Infant Causing Urinary Obstruction.** Hepler, A. B. [Seattle, Washington] *J. Urol.*, **49**:777-784. 1943.

A 3 week old male infant with urinary retention due to bladder displacement by a large presacral sympathicoblastoma is reported. The tumor was only partially removed and postoperative x-radiation was administered. The child is living and well 4 years later. These tumors arise embryologically from "migrating cells from the ganglionic crest of the medullary tube." The histologic structure indicates that they arise at various stages in the differentiation of the cells toward their normal adult status. The majority are highly malignant. A case reported by Cushing indicates that the tumor may spontaneously become more mature in type and thus change from a malignant to a benign state. Many of these tumors are extra-adrenal in origin.—V. F. M.

**Intracranial Tumors.** Jelsma, F. [Louisville, Ky.] *Kentucky M. J.*, **39**:203-208. 1941.

Symptoms resulting from intracranial tumors occurring in various portions of the brain are discussed. The symptomatology of the frontal, temporal, parietal, and occipital lobes, as well as basilar and cerebellar symptom complexes are illustrated by 8 case histories.—J. L. M.

**Centrally Located Tumors of the Frontal Parts of the Brain.** Kirschbaum, W. R. [Manteno State Hosp., Manteno, Ill.] *Illinois M. J.*, **83**:267-271. 1943.

Report of 3 cases. The author believes that studies of patients with frontal brain tumors are of value for the correlation of experimental work and neuropsychiatric observation and also will add to our present knowledge of the function of the frontal lobes.—M. E. H.

**Paraphysial Cysts of the Third Ventricle. Diagnosis and Surgical Management.** Weinberger, L. M., and Boshes, B. [Chicago, Ill.] *Surgery*, **13**:368-384. 1943.

Diagnosis and surgical management are discussed. A case is reported in which a paraphysial cyst was successfully removed, and 16 similar cases are reviewed.—W. A. B.

## Eye

**Epithelial Tumors of the Limbus.** Ash, J. E., and Wilder, H. C. [Army Med. Museum, Washington, D. C.] *Am. J. Ophth.*, **25**:926-932. 1942.

A general discussion.—E. C. R.

**Primary Sarcoma of the Choroid. Report of a Case.** Barlow, A. [Philadelphia, Pa.] *Am. J. Ophth.*, **25**:1337-1340. 1942.

A report of a case that was observed over a period of 20 years.—E. C. R.

**Five Hundred Melanomas of the Choroid and Ciliary Body Followed Five Years or Longer.** CALLENDER, G. R., WILDER, H. C., and ASH, J. E. [Washington, D. C.] *Am. J. Ophth.*, **25**:962-967. 1942.

A statistical study based on the specimens filed with the American Registry of Ophthalmic Pathology at the Army Medical Museum.—E. C. R.

**Adrenal Neuroblastoma with Orbital Metastases. A Case Report with Autopsy.** GUIBOR, G. P. [Children's Mem. Hosp., Chicago, Ill.] *Am. J. Ophth.*, **25**:387-397. 1942.

A case report.—E. C. R.

**Intraepithelial Epithelioma of the Cornea and Conjunctiva (Bowen's Disease).** MCGAVIC, J. S. [Inst. of Ophth., Presbyterian Hosp., New York, N. Y.] *Am. J. Ophth.*, **25**:167-176. 1942.

A general discussion with report of 5 cases. Ocular involvement by Bowen's disease has not previously been recorded.—E. C. R.

**The Early Diagnosis of Choroidal Melanomas.** RONES, B. [Washington, D. C.] *Am. J. Ophth.*, **25**:39-42. 1942.

A general discussion.—E. C. R.

**Teratoid Tumor of Conjunctiva and Other Developmental Anomalies with Naevus Verrucosus of Scalp.** SHERMAN, A. R. [Newark, N. J.] *Arch. Ophth.*, **29**: 441-445. 1943.

A case report.—E. C. R.

**Orbital Metastasis from Tumor of the Pancreas. Report of Two Cases with Necropsy Findings.** SNIDERMAN, H. R. [Cincinnati Gen. Hosp., Cincinnati, Ohio] *Am. J. Ophth.*, **25**:1215-1221. 1942.

A report of 2 cases.—E. C. R.

**A Case of Bowen's Disease of the Cornea.** WISE, G. [Inst. of Ophth., New York, N. Y.] *Am. J. Ophth.*, **26**:167-171. 1943.

Bowen's disease is an intraepithelial epithelioma which usually occurs on skin and mucous membrane. Recently it has been observed to involve the cornea. An additional case is reported.—E. C. R.

## EAR

**Osteoma of the External Auditory Canal.** DRUSS, J. G., and MAYBAUM, J. L. [Mt. Sinai Hosp., New York, N. Y.] *Arch. Otolaryng.*, **36**:499-509. 1942.

Three cases are reported in detail. A review of the literature is presented. Questions regarding the frequency of occurrence, origin, site of attachment, predisposing factors, microscopic appearance, and clinical course are considered. The indications for removal of these growths and the various operative procedures are discussed.—M. E. H.

**Metastatic Malignant Neoplasm of the External Auditory Canal and Middle Ear: Report of Case.** JOHNSON, H. R. [Fairmont, W. Va.] *West Virginia M. J.*, **37**: 128-130. 1941.

Tumors in this location, either primary or secondary, are rare. Pain is a prominent symptom. A mass may appear in the auditory canal, and spontaneous hemorrhage has occasionally been profuse. A few patients have seventh nerve paralysis. The patient described had secondary hypernephroma in the middle ear and auditory canal after removal of the kidney involved by the primary tumor.—E. E. S.

**Melanosis of the Internal Ear.** LEDERER, F. L. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Arch. Otolaryng.*, **35**:267-280. 1942.

No case of melanoma of the internal ear has been reported previously. Histologic examination of the tissues of the internal ear of a child who succumbed to melanocarcinomatosis of the dura permitted a study of the distribution of pigment in this region. A comparison between melanosis of the internal ear and melanosis oculi is drawn, and the essential differences discussed. The case gave an opportunity to study the biology of melanomas especially as they concern the internal ear.—M. E. H.

## BREAST

**Metastatic Tumors in the Breast.** CHARACHE, H. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Ann. Surg.*, **115**: 47-50. 1942.

The author emphasizes the rare occurrence of metastatic tumors in the breast. Two cases are added to the 10 previously reported in the literature.—M. R. D.

**Carcinoma of the Female Breast. An Analysis of the End Results after Five Years in One Hundred and Ninety-Two Cases, with Special Consideration of Preoperative Irradiation.** COHN, L. C. [Baltimore, Md.] *Arch. Surg.*, **44**:715-722. 1942.

Patients who received various types of treatment for carcinoma of the breast, including complete operation, complete operation with preoperative irradiation, irradiation with excision of tumor, and biopsy and excision only, were followed from 8 to 12 years. Analysis of the cases indicates that the end results have been largely determined by the grade of the tumor and the condition of the axillary nodes at the time of operation. In one group having irradiation and local excision or simple mastectomy, there was some evidence that irradiation therapy had influenced the end results. In 68 cases of recurrent or inoperable carcinoma the evidence indicates that the end results were not affected by irradiation therapy. In this group the 5 year cure rate was less than 1%.—R. C. R.

**Cancer of the Breast.** HEGNER, C. F. [Denver, Colo.] *Rocky Mountain M. J.*, **40**:241-244. 1943.

A general summary of cancer of the breast, discussing early diagnosis and present day treatment.—M. E. H.

**Sarcoma of the Breast.** HILL, R. P., and STOUT, A. P. [Coll. of Physicians and Surgeons, New York, N. Y.] *Arch. Surg.*, **44**:723-759. 1942.

It has been estimated that from 0.5 to 3.0% of all malignant tumors of the breast are sarcoma. In a series of tumors observed between 1911 and 1940, 15 were adenosarcoma, 5 fibrosarcoma, 2 lymphosarcoma, 2 leukemic tumors, 2 carcinosarcoma, and 2 malignant hemangioendothelioma. Osteoid or cartilaginous tissue found in 3 tumors was believed to represent a transition or metaplasia of the tumor elements to the more mature cell type. An attempt was made to separate the widely variable types of sarcoma into distinct histological groups. The various types and forms were discussed in some detail, and the literature was cited.—R. C. R.

**The Halsted Radical Mastectomy. Five-Year Results in 246 Consecutive Operations at the Same Clinic.** Hoopes, B. F., and McGraw, A. B. [Henry Ford Hosp., Detroit, Mich.] *Surgery,* **12**:892-905. 1942.

A summary of 240 cases in which radical mastectomy was done. Postoperative mortality was 2.8%. Five year survivals occurred in 71% of the patients with no axillary metastases and in 28% of those with axillary metastases. X-ray therapy did not improve the 5 year survival rate in patients without axillary involvement (no x-ray therapy: 74.4% of 43 patients survived 5 years or more; one course of x-ray therapy: 68.4% of 38 patients; two courses of x-ray therapy; 68.7% of 16 patients). However, x-ray treatment added slightly to the survival of patients with axillary metastases (no x-ray therapy: 21.8% of 55 patients; one course of x-ray therapy: 35.8% of 55 patients; two courses of x-ray therapy: 26.8% of 41 patients survived).—W. A. B.

**Chronic Mastitis and Carcinoma.** McKinley, D. [Seattle, Wash.] *West. J. Surg.,* **51**:234-239. 1943.

Chronic cystic mastitis predisposes to cancer. The risk is not sufficiently great to warrant bilateral mastectomy in all cases, but the patients should be followed with care. It is suggested that hormonal therapy or pregnancy may be permissible in patients under 30, though it should be realized that the results in the literature are thus far equivocal.—M. E. H.

**Radical Mastectomy for Carcinoma of the Breast.** Marshall, S. F. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North America,* **22**:721-735. 1942.

Radical mastectomy forms the principal basis for the present day method of treatment of cancer of the breast. The method employed for some years at the Lahey Clinic is in principle the Halsted operation, which has been modified in certain minor details. The technic of the operation is described in detail, and the indications and contraindications for its use are discussed.—J. L. M.

**Pregnancy Complicating Carcinoma of the Breast.** Power, H. A. [Pittsburgh, Pa.] *Pennsylvania M. J.,* **45**:1049-1050. 1942.

Carcinoma of the breast complicated by pregnancy is of infrequent occurrence. The best results are probably secured by treatment of the growth first and the pregnancy second. It is probably dangerous to permit subsequent pregnancy until at least 3 years have elapsed. The individual variation in the rate of tumor growth and in the rapidity and extent of metastases prevents the formation of definite rules of treatment and justifies any procedure aimed at increasing the margin of safety for the patient. Reports of 4 cases are presented.—J. L. M.

## Female Genital Tract

**Dermoid Cyst in the Uterine Tube.** Aaron, J. B. [Beth Israel Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.,* **42**:1080-1086. 1941.

A case report and review of the literature. The dermoid is said to have originated within the tubal mucosa.—A. K.

**A Clinicopathologic Study from Thirty-Two Cases of Chorioepithelioma.** Acosta-Sison, H., and Espaniola, N. [Coll. of Med., Univ. of Philippines, Manila, P. I.] *Am. J. Obst. & Gynec.,* **42**:878-884. 1941.

Thirty-two cases revealed the following findings. The ages of the patients ranged from 17 to 49 years. Chorionepithelioma was more common in multigravidas than primigravidas. It was more common after abortion than after full term labor, and especially common after mole expulsion. Symptoms indicative of chorionepithelioma occurred from the day after the termination of pregnancy to 3 years thereafter. Removal of the primary tumor did not bring about resorption of the metastases. In the presence of symptoms, negative x-ray findings did not exclude the presence of lung metastases. Surgical removal of a tumor in an accessible place and early x-ray treatment of the lungs if metastases were present proved to be the best form of therapy. The Friedman test as a diagnostic aid is evaluated.—A. K.

**Carcinoma of the Cervix Uteri.** Arneson, A. N. [St. Louis, Mo.] *J. Oklahoma State M. A.,* **34**:281-285. 1941.

The changes in cervical carcinoma, initiated by radiotherapy, such as tumor cell degeneration, invasion by phagocytes, and scar formation, are described. Radiosensitivity cannot be determined by histologic study alone. Infected tumors in general are more radioresistant, although necrosis and sloughing may develop early in the course of treatment. The tumor most sensitive to radiation is the bulky projecting lesion that is usually less invasive. The diffusely infiltrating tumor yields less readily to radiation, possibly because of preexisting fibrosis and ischemia. The tumor with a large ulcer crater is also radiosensitive despite the invariable presence of infection.

In the treatment of all types, x-rays should precede use of radium to reduce infection. Infection can also be reduced by vaginal irrigations. It is essential to distribute the necessary amount of radiation as evenly as possible through the tumor-bearing area.—E. E. S.

**Spontaneous Amputation and Subsequent Acute Torsion of the Normal Left Fallopian Tube and Fibroma of the Right Ovary.** Barrett, C. W., and Lash, A. F. [Univ. of Illinois, Chicago, Ill.] *West. J. Surg.,* **51**:135-139. 1943.

A case report. Fibroma of the right ovary was an incidental finding in this case, and the author briefly reviews the incidence and symptomatology of these tumors.—M. E. H.

**Myoma of the Vagina.** Bennett, H. G., Jr., and Ehrlich, M. M. [Johns Hopkins Univ., Baltimore, Md.] *Am. J. Obst. & Gynec.,* **42**:314-320. 1941.

Nine cases of primary myoma of the vagina were studied and the literature reviewed. It is concluded that the factors responsible for the appearance of uterine myomas do not affect the induction of myomas of the vagina any more than those of the gastrointestinal tract. Uterine myomas are more common in the colored than in the white population, whereas the situation is reversed in the case of myomas of the vagina. The occurrence of vaginal myomas seems not to be related to embryologic remnants such as Gartner's duct.—A. K.

**Basal Cell Carcinoma of the Vulva.** BERMAN, W. [Barnard Free Skin and Cancer Hosp. and Jewish Hosp., St. Louis, Mo.] *Am. J. Obst. & Gynec.*, **42**:1070-1076. 1941.

A report of 4 cases. One death was recorded as due to postoperative cardiac failure and pulmonary edema following a Bassett operation 2 years after the preliminary vulvectomy. There was no evidence of local recurrence. The other 3 patients are alive and well 8 years, 9 years, and 6 months respectively, after surgical extirpation of the tumors without irradiation. In 3 patients a regional lymph node was removed, but in no case was there evidence of metastasis.—A. K.

**Dermoid Cyst of the Ovary with Squamous Cell Carcinoma.** BRODY, S. [Beth Moses Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **41**:522-525. 1941.

An instance of primary carcinoma arising from the epithelial elements of a dermoid cyst.—A. K.

**The Role of Deep Cauterization in the Prevention of Cancer of the Cervix. A Report of Ten Thousand Cases.** CASHMAN, B. Z. [Univ. of Pittsburgh, Pittsburgh, Pa.] *Am. J. Obst. & Gynec.*, **41**:216-224. 1941.

The author concludes that chronic cervicitis is a contributing factor in the etiology of carcinoma of the cervix. Prevention and adequate treatment of cervicitis, and early diagnosis of the growth by periodic examination are said to offer at present the best solution of the problem of cervical cancer. Deep and extensive cauterization is often necessary to destroy infection in the cervix, with careful postoperative management to prevent subsequent stenosis of the cervical canal. Only 2 cases of cancer of the cervix occurred in 10,000 patients treated by deep cauterization of the cervix. A longer period of observation is necessary, however, to determine whether the incidence of cancer for this age group is actually being reduced.—A. K.

**Total Hysterectomy, Abdominal and Vaginal.** DANFORTH, W. C. [Evanston Hosp., Evanston, Ill.] *Am. J. Obst. & Gynec.*, **42**:587-598. 1941.

To avoid the danger of cancer in the retained stump and to free the patient from the annoyance that may be caused by the unhealthy stump, it is recommended that a total removal of the uterus be more frequently carried out. The operation may be either abdominal or vaginal, as the individual case may suggest. The gynecologist should be able to do either operation with sufficient ease so that his choice of operation is not influenced by his lack of experience. He should not choose the incomplete operation because he feels he can do it more easily. The experienced operator should be able to develop these operative technics readily. If proper technic is used there is no objection to complete removal of the uterus. Total removal in experienced hands does not subject the patient to increased risk. The operator of small experience in pelvic surgery should make use of the subtotal operation.—A. K.

**The Treatment of Pelvic Endometriosis.** DANNREUTHER, W. T. [New York Post-Graduate Med. Sch. and Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **41**:461-474. 1941.

Progesterone is said to have contributed to the comfort of some patients with endometriosis. Concurrent fibromyomatous tumors have been found in 40% of cases. The majority of cases of adenomyosis and adenomyoma are found in women of more than 35 years of age, and hysterectomy is necessary for cure. Intrauterine radium therapy may be effective in the treatment of adenomyosis, but the value of roentgen ray therapy in cases of adenomyoma and chocolate cysts is questionable. Complete ovarian extirpation is necessary for the cure of bladder and intestinal wall involvements.—A. K.

**Bilateral Ovarian Dermoid Cysts Complicating Pregnancy Treated by Bilateral Oophorectomy.** GOODWIN, R. H. [St. Luke's Hosp., New Bedford, Mass.] *Am. J. Obst. & Gynec.*, **44**: 525-527. 1942.

A case of bilateral dermoid cysts complicating pregnancy. Oophorectomy was performed during the first trimester. Delivery was at term. Menstrual-like bleeding occurred for 3 days, 6 months postpartum, though the endometrium proved to be atrophic. An otherwise typical surgical menopause followed.—A. K.

**Local Refrigeration in Genital Cancer. A Report of Ten Cases.** GORDON, C. A., and CRESCI, J. V. [Kings County Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **41**: 281-285. 1941.

Ten patients with advanced genital cancer, 9 with carcinoma of the cervix, and 1 with carcinoma of the vulva received local refrigeration therapy. There was notable diminution in size of the tumor in 3 instances, relief of pain in 2, pronounced necrosis of the cancerous refrigerated area in 3, and cellular degenerative changes in 1 case. Death occurred in coma in 4 cases.—A. K.

**When Are Gynecological Operations Indicated?** HEANEY, N. S. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Surg. Clin. North America*, **23**:1-9. 1943.

A discussion of the indications for gynecological operations, with a description of ovarian tumors.—J. L. M.

**The Incidence of Endometrial Hyperplasia with Uterine Fibroids and External and Internal Endometriosis (Adenomyosis).** HENDERSON, D. N. [Univ. of Toronto, Toronto, Canada] *Am. J. Obst. & Gynec.*, **41**:694-697. 1941.

It is concluded that disturbance in estrin production is not of etiologic significance in fibroid tumors and external endometriosis, although it may be in adenomyosis. Endometrial hyperplasia was found in 25% of 82 cases of adenomyosis.—A. K.

**A Study of the Ovaries and Endometriums of Patients with Fundal Carcinomas.** JONES, H. O., and BREWER, J. I. [St. Luke's Hosp. and Northwestern Univ. Med. Sch., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **42**:207-217. 1941.

The etiologic significance of hyperestrinism or the unopposed action of estrin in endometrial carcinoma was not demonstrated, according to the authors, in the patients studied for this report. In premenopausal patients with carcinoma, the corpora lutea were normal as was the uterine endometrium not involved in the malignant change. Cystic glandular hyperplasia was not present in any of the patients with cyclic menstruation. Cystic ovarian changes were considered incidental and without significance.—A. K.

**Arrhenoblastoma of the Ovary.** KANTER, A. E., and RAGINS, A. B. [Cook County Hosp., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **42**:1061-1065. 1941.

A report of a case characterized clinically by symptoms of defeminization and masculinization. It is stated that the

diagnosis of arrhenoblastoma of the ovary is greatly facilitated by the use of the fluorescence microscope, which allows a more definite demonstration of the cells resembling the testicular Leydig cells.—A. K.

**Krukenberg's Tumors.** Leffel, J. M., Jr., Mason, J. C., and Dockerty, M. B. [Mayo Clinic, Rochester, Minn.] *Ann. Surg.*, **115**:102-113. 1942.

A survey of 44 cases of Krukenberg's tumors encountered at the Mayo Clinic from 1908 to 1938 inclusive. The histogenesis, clinical and pathologic observations, routes of metastases, and surgical treatment are discussed.—M. R. D.

**Dermoid Cysts of the Ovary.** Linn, H. J., and Ragins, A. B. [Cook County Hosp., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **41**: 328-330. 1941.

The histories and pathological reports of 103 cases of dermoid cysts of the ovary were studied. It is concluded that dermoid cysts are seldom diagnosed preoperatively because of the indefiniteness of the symptoms. Often they are incident to other pelvic conditions.—A. K.

**Endometrial Polypoid Fibrosarcoma.** Lomax, J., and Anderson, G. [Buffalo Gen. Hosp. and Univ. of Buffalo Med. Sch., Buffalo, N. Y.] *Am. J. Obst. & Gynec.*, **42**:1049-1057. 1941.

A case report. Although this type of sarcoma is an actively proliferative, true sarcoma of fibroblastic type with a tendency to the formation of giant cells, it has also a very definite tendency to fibrotic regression. Total extirpation of the uterus may provide adequate therapy.—A. K.

**Benign Tumors of Vulva.** Lovelady, S. B., McDonald, J. R., and Waugh, J. M. [Mayo Clinic, Rochester, Minn.] *Am. J. Obst. & Gynec.*, **42**:309-313. 1941.

Thirty-four benign vulval neoplasms were studied. Fibromas, lipomas, and hemangiomas were the commonest types in the order named. Two neurofibromas, two leiomyomas, a ganglioneuroma, and a lymphangioma were encountered. In the majority of cases there were no symptoms.—A. K.

**Pubertas Praecox Due to Ovarian Tumors.** Lull, C. B. [Philadelphia Lying-In Hosp., Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **41**:445-454. 1941.

Two cases of ovarian tumor associated with precocious puberty are reported. One of the growths was a typical granulosa cell tumor, the second a simple follicular cyst. Both patients returned to the normal infantile type after surgical removal of the lesion. The fact that the second case responded to operative procedure suggests that surgery is indicated in these ovarian tumors even though they are not of granulosa cell type.—A. K.

**Adenocarcinoma of the Vulva.** McDonald, J. R., Lovelady, S. B., and Waugh, J. M. [Mayo Clinic, Rochester, Minn.] *Am. J. Obst. & Gynec.*, **42**:304-309. 1941.

A report on 32 cases. Thirty tumors were papillary adenocarcinomas that originated from the apocrine sweat glands. One was a nonpapillary adenocarcinoma, and one an adenocarcinoma of the cylindroma type. The papillary carcinomas did not show a tendency to metastasize and were cured by simple excision.—A. K.

**Hydatidiform Mole Followed by Chorionepithelioma.** McLaughlin, E. F. [Germantown Hosp., Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **42**:904-907. 1941.

A report of two cases of hydatidiform mole followed by chorionepithelioma. In each instance hysterectomy was followed by recovery.—A. K.

**The Diagnostic Value of Vaginal Smears in Carcinoma of the Uterus.** Papanicolaou, G. N., and Traut, H. F. [Cornell Univ. Med. Coll. and New York Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **42**:193-206. 1941.

The method of taking and staining vaginal smears is described. Findings in smears diagnostic of carcinoma of the cervix and fundus of the uterus are given. The method is said to yield a high percentage of correct diagnoses as checked by tissue biopsies. It is felt by the authors that a positive diagnosis may be obtained in some cases of early disease. The simplicity of the method, the lack of inconvenience to the patient, and the possibility of obtaining daily information over a long period of time should make this procedure very useful in following the progress of the disease after operative procedures or x-ray treatments. Interpretation of the smear requires a pathologist or cytologist who has had considerable experience with the technic.—A. K.

**Bilateral Brenner Tumor of the Ovary.** Peale, A. R. [Temple Univ. Hosp. and Sch. of Med., Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **44**:138-141. 1942.

A case of bilateral Brenner tumor in a 50 year old female, complicated by strangulation of the right ovarian mass through torsion of the pedicle.—A. K.

**Adenomyosis Interna Uteri as a Cause of Uncontrollable Atony and Hemorrhage Following Cesarean Section for Sacculation Pregnancy. Hysterectomy.** Sackett, N. B. [Woman's Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **42**:894-898. 1941.

A report of repeated elective cesarean section because of uterine atony. The latter was probably caused by widespread adenomyosis of the myometrium.—A. K.

**Leiomyomyxosarcoma of the Uterus.** Simon, H. J. [City Hosp., Welfare Island, New York, N. Y.] *Am. J. Obst. & Gynec.*, **41**:339-340. 1941.

Report of a case of multiple myomas and multiple leiomyosarcomas of the uterus with peritoneal sarcomatosis. —A. K.

**Solid Teratoma of the Ovary in the Young Girl.** Smeltzer, M. [Henry Ford Hosp., Detroit, Mich.] *Am. J. Obst. & Gynec.*, **41**:616-623. 1941.

Two cases of teratoma are reported and the literature is reviewed. Solid ovarian teratoma in the ovary of the young girl is extremely malignant. Cure depends upon complete surgical removal. The author's experience with x-ray treatment of this tumor has not been encouraging.—A. K.

**A Rare Connective Tissue Tumor (Myxosarcoma) in the Area of the Bartholin Gland.** Smiley, I., and Kushner, J. I. [Bronx Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **44**:709-711. 1942.

A case in which a slowly growing myxosarcoma presented the clinical picture of a retention cyst of Bartholin's gland.—A. K.

**Nationality and Carcinoma of the Cervix.** SMITH, F. R. [Memorial Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **41**:424-430. 1941.

In the Gynecological Clinic at Memorial Hospital the incidence of carcinoma of the cervix has been relatively low in Jewish women and high among the Italian and Scotch-English in contrast with the incidence in other nationalities. No adequate explanation for these differences has been found.—A. K.

**Chorioepithelioma. Associated with Tubal Pregnancy and Pulmonary Metastases. High Gonadotropin and Low Pregnanediol Values.** SMITH, J. T., and WERTHESSEN, N. T. [Joseph H. Pratt Diagnostic Hosp., Boston, Mass.] *Am. J. Obst. & Gynec.*, **41**:153-156. 1941.

A case is presented of chorionepithelioma that followed a left tubal pregnancy, causing almost none of the usual signs and symptoms, and spreading by metastasis to the lungs before it was recognized locally. Hormone assays showed high chorionic gonadotropin and low pregnanediol values.—A. K.

**Myoblastoma of Labium Majus.** TAMIS, A. B., and KOWLES, J. J. [Lebanon Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **42**:543-544. 1941.

An unusual tumor of the labium majus, having the appearance of a myoblastoma, is described. The mass was removed and has not recurred in 6 months of observation. Other cases reported since 1926 indicate the benign nature of the tumor.—A. K.

**Double Primary Ovarian Malignancy. Granulosa Cell Tumor of the Ovary Following Radium Therapy for Cervical Carcinoma with Papillary Cystadenoma of the Same Ovary.** TYRONE, C. H., and WEED, J. C. [Tulane Univ. Sch. of Med. and Touro Infirmary, New Orleans, La.] *Am. J. Obst. & Gynec.*, **42**:147-150. 1941.

A report of granulosa cell tumor of the ovary following radium therapy for cervical carcinoma with papillary cystadenoma of the same ovary. The authors do not feel that the evidence is conclusive, but suggest the possibility of irradiation as an inciting factor in ovarian malignancy.—A. K.

**The Role of the Fallopian Tubes in the Spread of Pelvic Cancer. Report of a Case with a Brief Review of the Literature.** WALLIS, O. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **41**:196-206. 1941.

The presence of free cancer particles in the normal fallopian tube may indicate the pathway of spread of pelvic cancer. A case is reported illustrating this theory.—A. K.

**The Diagnosis and Treatment of Carcinoma of the Corpus Uteri Based on Experiences at the Woman's Hospital.** WARD, G. G. [Woman's Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **44**:303-309. 1942.

Early diagnosis gives the best chance for cure. Diagnostic curettage is most important in all cases of persistent bleeding. The prognosis is better when the uterus is small or normal sized. Ultimate prognosis depends upon age, physical state of the patient, duration of symptoms, extent of disease, prompt diagnosis, and type of treatment. Diagnostic curettage and intracavitary irradiation, total hysterectomy and bilateral salpingo-oophorectomy, and deep x-ray therapy are the ideal treatment. This treatment for operable lesions should give on the average a 70%, 5 year survival rate; for inoperable lesions, a 35% rate. Irradiation alone has a definite value in producing cures. The early prompt removal of the uterus, tubes, and ovaries is the most essential factor in the treatment of carcinoma of the corpus, irradiation being a valuable adjunct.—A. K.

**Carcinoma of Cervical Stump.** WATKINS, R. E. [Univ. of Oregon Med. Sch., Portland, Oreg.] *Northwest Med.*, **40**: 172-176. 1941.

Those cancers appearing within 1 year of hysterectomy were regarded as persistent cancer of the cervical stump. If the cervical cancer was recognized later than 1 year after operation, it was thought to be a primary, or true, stump cancer. Total hysterectomy is not advised as a substitute for subtotal resection of the uterus because of the higher mortality rate following total removal. However, it is considered the operation of choice when there is any accompanying lesion of the cervix, unless the patient is a poor operative risk. Every effort should be made to examine the cervical stump carefully at the time of operation. Surgical removal of the stump carcinoma is not as efficacious as radium implantation and high voltage roentgen irradiation. The prognosis is generally good.—E. E. S.

**Theca Cell Tumors of the Ovary.** WOLFE, S. A., and NEIGUS, I. [Long Island Coll. Hosp., and Brooklyn Jewish Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **42**:218-228. 1941.

Eight cases of theca cell tumor are reviewed. Associated uterine pathologic conditions were found. Amenorrhea or atypical menstrual bleeding was the predominant complaint. The authors feel that these tumors are related histogenetically to granulosa cell tumors. Either an insular or diffuse yellow appearance on cut section is characteristic. Spindle, fusiform, or polygonal cells surrounded by fine connective tissue fibrils are found microscopically. Intracellular lipids demonstrable microscopically are characteristic. This lipid material is related to the biological activity of the tumor.—A. K.

**Bilateral Granulosa Cell Tumors. Successful Removal from a Child Fourteen Weeks of Age.** ZEMIKE, E. E., and HERRELL, W. E. [Mayo Clinic, Rochester, Minn.] *Am. J. Obst. & Gynec.*, **41**:704-707. 1941.

Bilateral tumors of the ovaries were discovered in a child 14 weeks of age, and the tumors were successfully removed when she was 16 weeks old. This is the youngest patient recorded with this type of tumor. Three years and two months after operation the patient is in good health. In only 5 to 10% of the cases do these tumors appear before puberty. Bilaterality does not occur in more than 5% of granulosa cell tumors.—A. K.

## MALE GENITAL TRACT

**Embryonal Carcinoma of the Testicle. Report of a Case in an Infant and a Review of the Recent Literature from a Clinical-Pathologic Viewpoint.** BRIELE, H. A. [Univ. of Maryland Med. Sch., Baltimore, Md.] *Bull. Univ. Maryland School Med.*, **27**:146-158. 1943.

The author presents a case occurring in an infant, and a review of the recent literature from a clinical-pathologic viewpoint. Emphasis is placed on the importance of an early diagnosis, and orchiectomy followed by adequate

deep roentgen therapy to all lymph node areas. The Friedman test, if positive, is of considerable help in judging the prognosis and supervising the treatment.—J. L. M.

**Hormones and Carcinoma of the Prostate.** Creevy, C. D. [Univ. of Minnesota, Minneapolis, Minn.] *Journal-Lancet,* **62**:452-454. 1942.

A short review of present methods of treatment of carcinoma of the prostate. On the basis of 60 cases, the author recommends that estrogens be tried in every case and castration resorted to only in those instances where estrogens have failed.—M. E. H.

**Tumors of the Testis.** Day, M. L. [Grunow Mem. Clinic, Phoenix, Ariz.] *Southwestern Med.,* **26**:408-410. 1942.

This review is concerned with the origin and classification of tumors of the testis, their diagnosis, and their treatment. The necessity for early diagnosis and treatment is stressed. Adequate radiation therapy is of great importance.—J. L. M.

**Pathology of Tumors of the Testis: Correlation with Excretion of Hormone in the Urine.** Ferris, D. O. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.,* **16**:615-621. 1941.

A study was made of all malignant tumors of the testes in cases encountered at the Mayo Clinic from 1930 to 1939 inclusive, in which the tumor was removed before roentgen treatment had been given and in which gonadotropic studies had been made.

Tumors of the testis are rare, and almost all are malignant, being either seminoma or teratoma. All malignant tumors of the testis cause excretion of excessive amounts of gonadotropic hormone in the urine. Twenty rat units per liter of urine is the dividing point between normal and excessive amounts. The test must be quantitative and the best technic is that of Frank. The routine on the first analysis is to test for 25, 40, and 66 rat units of hormone per liter of urine.—J. L. M.

**The Pathology of Carcinoma of the Prostate Gland: A Review.** Gordon, H. [Univ. of Louisville Sch. of Med., Louisville, Ky.] *Urol. & Cutan. Rev.,* **45**:646-654. 1941.

The death rate from cancer of the prostate has increased steadily and will probably continue to do so, since the proportion of males past 60 years of age is expected to increase. Approximately 17% of glands removed from middle aged males at autopsy or operation shows carcinoma. Some endocrine influence is likely, but the theories on this point conflict. The lesion most commonly arises in the posterior or lateral lobes. Induration and fixation are late signs. When the lesion is found early, surgical extirpation gives good results. Opinions differ widely on the value of radiation, but the author seems to think proof of generally good results from this treatment is lacking. The prognosis is poorest with the younger patients and with the most anaplastic types of growth.—V. F. M.

**Cancer of the Prostate in Connecticut.** Griswold, M. H. [Connecticut State Dept. of Health, Hartford, Conn.] *Connecticut M. J.,* **7**:499. 1943.

A report on the number of patients hospitalized for carcinoma of the prostate in the State since 1935, with the mortality figures.—M. E. H.

**Thoughts and Observations on the Relation of Altered Hormonal Balance to Prostatic Malignancy.** Herbst, W. P. [Washington, D. C.] *Urol. & Cutan. Rev.,* **46**:691-694. 1942.

The author traces the development of the endocrine therapy of prostatic cancer. The structural chemical relationship of sex hormones to cholesterol is illustrated and shown to be close. The liver contains much cholesterol and thus may play an important, though yet unknown, role. Luden is cited as suggesting that a low cholesterol diet tends to retard the growth of carcinoma, while a high cholesterol diet is definitely stimulating. A low cholesterol diet is therefore suggested as a further aid in the treatment of prostatic cancer. Twenty-seven cases treated with various combinations of endocrine therapy indicate that estrogens will be effective in those patients who are, as a rule, improved by castration. Where castration fails, estrogens are likewise ineffective. However, estrogens may sometimes continue the benefits of castration when the originally good response seems to be wearing off. Castration has proved effective with some patients who did not respond favorably to estrogens. The total amount of urine excreted by patients taking estrogens is greatly reduced in many instances and this seems partly to explain the frequent reduction in nocturia. The author urges the use of smallest estrogenic dosage that will give improvement, because of the possibility of a general hormonal derangement and of side effects such as the purpuric syndrome that developed in 2 of the author's patients.—V. F. M.

**Serum Acid Phosphatase Determination in Carcinoma of the Prostate.** Herger, C. C., and Sauer, H. R. [New York State Inst. for Study of Malignant Diseases, Buffalo, N. Y.] *Urol. & Cutan. Rev.,* **45**:283-285. 1941.

From a study of the acid phosphatase determinations on 125 patients, the authors conclude that pathologic levels obtain in the majority of cases of prostatic carcinoma with bone metastases. They advocate repeated determinations in doubtful cases, since an increasing or decreasing reading was found of even more importance than a single determination.—V. F. M.

**A Summary of Endocrine Effects in Advanced Prostatic Cancer.** Huggins, C. [Univ. of Chicago Sch. of Med., Chicago, Ill.] *Connecticut M. J.,* **7**:90-92. 1943.

The inhibition of androgens by estrogens in prostatic cancer as opposed to suppression by surgical castration is discussed. The author feels that estrogen inhibition is incomplete and temporary, and notes that estrogen must be administered for long periods of time. Therefore, bilateral orchiectomy is the method of choice, as complete inhibition or elimination of androgens is the basis for the modern treatment in advanced or metastatic prostatic cancer.—M. E. H.

**Malignant Priapism. Report of Primary Carcinoma of the Urethra with Priapism.** Ikeda, K., Foley, F. E. B., and Rosenow, J. [Miller Hosp., St. Paul, and Univ. of Minnesota, Minneapolis, Minn.] *J. Urol.,* **49**:732-744. 1943.

The literature is reviewed, and one new case is described in detail. Priapism was the result of infiltration of the corpora by tumor. The diagnosis was primary carcinoma of the urethra with invasion of penis and prostate and distant metastases.—V. F. M.

**The Treatment of Carcinoma of the Prostate Gland with Diethylstilbestrol and Diethylstilbestrol Dipropionate. An Analysis of Thirteen Cases.** KAHLE, P. J., OGDEN, H. D., JR., and GETZOFF, P. L. [Louisiana State Univ. and Charity Hosp., New Orleans, La.] *Urol. & Cutan. Rev.*, **46**:619-623. 1942.

Thirteen cases of carcinoma of the prostate treated with estrogens are described. Four patients died of nonneoplastic conditions, the remainder all improved. Repeated biopsies were made in 9 instances, and 8 showed histological change interpreted as due to estrogenic therapy. These changes are illustrated and consisted in the main of regressive nuclear alteration and vacuolization of the cytoplasm. The final stage showed clear acinar spaces containing pyknotic nuclei. No side effects of the therapy were noted except mild breast enlargement.—V. F. M.

**Treatment of Carcinoma of the Prostate with Estrogens.** KEARNS, W. M. [Milwaukee, Wis.] *Wisconsin M. J.*, **41**:575-581. 1942.

The author reports the results of treatment with estrogens of 37 patients with carcinoma of the prostate gland. Beneficial effects were obtained in a large majority of patients treated with estradiol and stilbestrol. The author feels that safety, economy, and comparable results give preference to the administration of estrogens over the method of castration.—M. E. H.

**The Hormonal Treatment of Prostatic Hypertrophy and Cancer. A Review and Initial Clinical Experiences.** KEYSER, L. D. [Roanoke, Va.] *Virginia M. Monthly*, **69**:544-550. 1942.

Surgery is still the method of choice for treating prostatic hypertrophy. In the treatment of nonoperable prostatic cancer, endocrine therapy has been more than encouraging.—M. E. H.

**Androgen Inhibition in the Treatment of Carcinoma of the Prostate.** LOW, H. T., and COAKLEY, H. E. [Pueblo, Colo.] *Rocky Mountain M. J.*, **39**:687-688. 1942.

The authors feel that while their series of 5 cases is much too small to be accepted as proof, androgen inhibition has much to offer in the treatment of carcinoma of the prostate and its metastases.—M. E. H.

**Primary Carcinoma of the Seminal Vesicles—Report of Two Cases—Review of the Literature.** MCCREA, L. E. [Temple Univ. Med. Sch., Philadelphia, Pa.] *Urol. & Cutan. Rev.*, **46**:700-703. 1942.

Nineteen instances of primary carcinoma of the seminal vesicles have been reported, to which the author adds 2. Both patients had urinary symptoms, and neither could be operated upon. Diagnosis was confirmed by biopsy through the rectum. Histologically the lesions were similar, being adenocarcinoma.—V. F. M.

**Tumors of the Epididymis and Tunica Vaginalis; Report of Two Cases.** MACKAY, C. H. [Los Angeles, Calif.] *J. Urol.*, **49**:440-443. 1943.

In 2 patients asymptomatic small masses intimately associated with the epididymes were found to be exactly alike histologically. After reviewing the literature, the author finds these tumors similar to others reported as lymphagioma, adenoma, and carcinoma. He, however, believes they are derived from the cells lining the serous cavity and thus are mesotheliomas of the tunica vaginalis. Communication of the tumor spaces and the tunica vaginalis cavity was found in one instance.—V. F. M.

**The Assay of Urinary Gonadotropin in Cases of Testicular Tumor.** MAIN, R. J. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **68**:381-384. 1941.

Brief mention is made of those conditions in which urinary gonadotropin is found. Some recommendations are made concerning the technic of assaying this hormone in cases of testicular hormone-producing tumors.—M. E. H.

**The Clinical Use of Peroral Methyl Testosterone in Benign Prostatic Hypertrophy.** MARKHAM, M. J. [Bronx, N. Y.] *Urol. & Cutan. Rev.*, **46**:225-227. 1942.

This is a clinical report of improvement in 4 of 6 cases of benign prostatic hypertrophy treated orally with methyltestosterone. No undesirable effects were noted.—V. F. M.

**Treatment of Prostatic Carcinoma by Castration or the Administration of Estrogen.** MARQUARDT, C. R. [Milwaukee, Wis.] *Wisconsin M. J.*, **41**:401. 1942.

Androgen suppression by castration or stilbestrol administration was tried in 33 patients with prostate carcinoma. The authors do not feel that either form of therapy results in cure, but both greatly improve and benefit the majority of cases of advanced carcinoma of this organ.—M. E. H.

**Carcinoma of the Prostate Gland with Special Reference to Endocrine Treatment.** MARQUARDT, C. R., and FLAHERTY, W. A. [Marquette Univ., Milwaukee, Wis.] *Urol. & Cutan. Rev.*, **46**:343-346. 1942.

The authors do not find the acid and alkaline phosphatase determinations to be of great aid in evaluating cases of prostatic carcinoma. They suggest that this may be due in part to technical difficulties with the test. Following endocrine therapy the phosphatase studies were "not conclusive." This does not tally with Huggins' descriptions. From their series of 33 patients it is concluded that endocrine therapy decidedly improves about 70%. Their impression is that none of the patients were cured. —V. F. M.

**Sarcoma of the Prostate Gland: Review of Literature; Table of Classification; Report of Four Cases.** MELICOW, M. M., PELTON, T. H., and FISH, G. W. [Presbyterian Hosp., New York, N. Y.] *J. Urol.*, **49**:675-707. 1943.

This is a comprehensive article. The literature is reviewed. In all approximately 200 instances of sarcoma of the gland are reported. A classification of the tumors of the prostate is included. The principal types of sarcoma are: fibrosarcoma, myosarcoma, and lymphosarcoma, which are termed "isoplastic." Seventy-five per cent of the cases occur in persons less than 40 years of age. Serum acid phosphatase is not elevated in this disease even when metastases have occurred. The prognosis is very poor, but old patients appear to live somewhat longer than younger ones. Treatment consists of surgical intervention, either alone or combined with irradiations. If the disease has spread beyond the capsule prostatectomy is contraindicated. With leiomyosarcoma limited to the prostate, radical prostatectomy may offer hope. The best results in general

are obtained from radiation. Four cases are reported: 2 of fibrosarcoma, 1 of lymphosarcoma, and 1 of rhabdomyosarcoma. All patients died.—V. F. M.

**Dosificación de la fosfatasa ácida en los enfermos de carcinoma de la próstata. [Determination of Acid Phosphatase in Patients with Carcinoma of the Prostate.** Mena Brito, M. A. ["Hospital General," Mexico D. F., Mexico] *Prensa méd. Mexicana,* No. 7:127. 1942.

It was found that in patients with carcinoma of the prostate the acid phosphatase in 100 cc. of blood was about 20 units higher than in normal persons. These figures are similar to those of Huggins and Hodges.—M. D-R.

**Orchidectomy in Prostatic Cancer. A Case Report.** Pernworth, P. H. [Venice, Ill.] *Illinois M. J.,* **83**: 338-339. 1943.

A case report. The author feels that, for the present, bilateral orchiectomy and the administration of stilbestrol to further lower the androgen concentration give the best therapeutic results in carcinoma of the prostrate with diffuse metastases.—M. E. H.

**Subcapsular Castration for Carcinoma of Prostate.** Riba, L. W. [Chicago, Ill.] *Proc. Inst. Med. Chicago,* **14**:273-274. 1942.

The subcapsular technic for removal of testicular parenchyma is simple, effective, and practical in the treatment of carcinoma of the prostate.—M. E. H.

**Prostatic Surgery at the Wisconsin General Hospital, 1940–1941.** Sisk, I. R., and Cornell, P. M. [Univ. of Wisconsin Med. Sch., and Wisconsin Gen. Hosp., Madison, Wis.] *Wisconsin M. J.,* **42**:679-683. 1943.

Of 421 patients 87 (20.6%) had carcinoma of the prostate. The average age was 69.7 years. Fifty-eight patients had transurethral resection (and castration in many instances), and 4 had radical perineal prostatectomy. The operative mortality was 1.7%.—M. E. H.

**Recent Trends in the Care of Prostatic Malignancy.** Stone, E. [Providence, R. I.] *Rhode Island M. J.,* **25**:106. 1942.

A brief discussion of the present available methods of treatment and a plea for early diagnosis by the family physician.—M. E. H.

**Carcinoma of the Prostate: A Study of 429 Cases.** Strohm, J. G. [Portland, Oreg.] *Urol. & Cutan. Rev.,* **45**: 770-771. 1941.

This is mainly a tabulation of the symptoms, age incidence, consistency of the tumor, and the lobes involved. The author states that one-fifth of patients seeking relief from bladder neck obstruction have prostatic carcinoma. Ninety per cent are incurable when first seen.—V. F. M.

**The Sedimentation Rate in Cases of Benign Hypertrophy and Carcinoma of the Prostate Gland and Carcinoma of the Prostate Gland with Metastasis.** Tillisch, J. H., and Habein, H. C. [Mayo Clinic, Rochester, Minn.] *J. Urol.,* **49**:857-860. 1943.

The authors tested the sedimentation rate with the Westergren technic in 100 cases each of benign prostatic hypertrophy, carcinoma of the prostate without evident metastases, carcinoma of the prostate with metastases (proved by biopsy). The average sedimentation rates were 25.06 mm., 38.26 mm., 61.50 mm. respectively. Twenty-three per cent of the patients with carcinoma with metastases did have sedimentation rates within the arbitrarily chosen normal (30 mm.), whereas 72% of those with benign hypertrophy had rates within this figure. The authors thus suggest the sedimentation rate is a valuable additional aid in determining presence or absence of metastases. —V. F. M.

**Treatment of Carcinoma of the Prostate with Estrogens.** Unger, A. [Veterans' Administration, Huntington, W. Va.] *M. Bull. Vet. Admin.,* **19**:448-449. 1942-43.

Early results following injections of diovocyclin appear promising.—M. E. H.

**Algunas consideraciones sobre el diagnóstico y tratamiento del carcinoma de la prostata. [Some Considerations on Diagnosis and Treatment of Cancer of the Prostate.]** Villanueva, A. [Hospital General, Mexico, D. F.] *Medicina,* **22**:307-317. 1942.

A review of recent advances in the treatment of the condition and an analysis of 100 cases observed by the author.—M. D-R.

**Stilbestrol in Prostatic Carcinoma. Effect on Metastasis.** Weller, C. G. [Chicago, Ill.] *Proc. Inst. Med., Chicago,* **14**:188-189. 1942.

Report on the progress of 10 patients with prostatic carcinoma treated with diethylstilbestrol orally for 3 to 6 months. All showed improvement.—M. E. H.

**Sarcoma of the Penis.** Wheelock, M. C., and Clark, P. J. [New England Deaconess Hosp., Boston, Mass.] *J. Urol.,* **49**:478-481. 1943.

Three cases of penile sarcoma are described and illustrated, one being a fibrosarcoma, 2 being malignant melanomas.—V. F. M.

**Orchidectomy as a Therapeutic Measure in Prostatic Carcinoma.** Editorial. *Rocky Mountain M. J.,* **39**:333. 1942.

The experience of the writer in a small series of cases has convinced him that Huggins has made a memorable contribution to therapeutics by advocating castration in prostatic carcinoma.—M. E. H.

## Urinary System—Male and Female

**Metastatic Malignant Tumors of the Kidney: A Review of the Literature and Report of 23 Cases.** Abeshouse, B. S., and Goldstein, A. E. [Sinai Hosp. and Univ. of Maryland Sch. of Med., Baltimore, Md.] *Urol. & Cutan. Rev.,* **45**:163-186. 1941.

This is a comprehensive article on the subject of renal metastases from lesions in other organs or the opposite kidney. A statistical analysis is made of 1,481 cases, including 23 personal ones. The authors conclude that renal metastases are relatively uncommon; that sarcoma metastasizes to the kidneys more readily than carcinoma; the greatest number of cases are associated with primary renal neoplasms; and that the usual signs of renal tumor are, as a rule, absent. One interesting conclusion seems worth quoting: "Notwithstanding the paucity of statistical reports on renal metastases associated with the various primary neoplasms of the body, we are inclined to agree with Albaman and Guillet that metastatic malignant disease

of the kidney is more frequently observed than primary neoplasms of the kidney."—V. F. M.

**Primary Carcinoma in a Diverticulum of the Bladder; a Report of Four Cases and a Review of the Literature.** Abeshouse, B. S., and Goldstein, A. E. [Sinai Hosp., Baltimore, Md.] *J. Urol.*, **49**:534-557. 1943.

From the literature 93 instances of primary carcinoma in a diverticulum have been gathered. Four cases are described and illustrated. The most common type of tumor is carcinoma, either squamous cell or papillary. Some of the neoplasms, however, are benign. Excision has not been satisfactory but is much better than nonoperative treatment. The majority of patients succumb within 2 years. —V. F. M.

**Sarcoma of the Kidneys in an Adult.** Davidson, B. [Beth Moses Hosp., Brooklyn, N. Y.] *Urol. & Cutan. Rev.*, **46**: 689-691. 1942.

This is a report of a 70 year old woman with unexplained fever, who was found to have a renal lesion. At autopsy, the lesion proved to have involved the left kidney and adjacent structures, and to have metastasized. Histologically the tumor was composed of a "cytoplasmic and argyrophilic reticulum." The diagnosis was reticulum cell lymphosarcoma. The exact origin of the tumor was obscure.—V. F. M.

**Benign and Malignant Lesions of the Urachus. With Special Reference to the Colloid Carcinoma of the Bladder.** Dreyfuss, M. L. [Chesapeake and Ohio Hosp., Clifton Forge, Va.] *Virginia M. Monthly*, **70**:200-204. 1943.

The author reviewed the embryology of the urachus and classifies the pathological conditions arising from it.—M. E. H.

**Metastasis of Carcinoma of Kidney to Heart and Thyroid. Report of a Case.** Dublin, W. B. [Western State Hosp., Fort Steilacoom, Wash.] *Northwest Med.*, **40**:215-216. 1941.

The tumor was a papillary adenocarcinoma of the kidney with metastases in many parts of the body including secondary lesions in the heart and thyroid.—E. E. S.

**Primary Carcinoma of the Male Urethra. (Report of One Case.)** Herger, C. C., and Sauer, H. R. [State Inst. for Study of Malignant Diseases, Buffalo, N. Y.] *Urol. & Cutan. Rev.*, **46**:346-348. 1942.

The authors present a brief review of the literature and an additional case. They point out that diagnosis is often difficult, particularly as about half the tumors are in the posterior portions of the urethra, and frequently there is a previous history of stricture. Their case illustrated these features. X-radiation did not affect the outcome of the disease. Most of the recorded cases were squamous cell carcinoma, but their patient had adenocarcinoma—apparently the third reported. Extensive resection offers the best hope of recovery. Biopsy of atypical urethral strictures is advocated.—V. F. M.

**Metastatic Renal Adenocarcinoma: Case Report.** Lane, C. [Santa Monica, Calif.] *Urol. & Cutan. Rev.*, **46**:7-8. 1942.

This is a complete case report, including autopsy, in which metastasis of a renal adenocarcinoma to the opposite kidney is recorded.—V. F. M.

**The Clinical Approach to Cortical Renal Neoplasms.** LeComte, R. M. [Washington, D. C.] *J. Urol.*, **49**:224-227. 1943.

The author discusses the classical triad of symptoms of renal neoplasm: lumbar pain, lumbar tumor, and hematuria. He points out that the presence of all 3 usually indicates a late state of the disease with a correspondingly smaller chance of cure. Pyelographic changes are also discussed. "Evidently hypernephroid growths may be present and remain benign for a while and then take on malignant characteristics." The early benign lesion is to be sought since here the best therapeutic results will be obtained.—V. F. M.

**Hemangioma of the Kidney.** McLean, E. H., and Mathews, T. J. [Oregon City, Oreg.] *West. J. Surg.*, **50**: 47-50. 1942.

A report of an unusual case in a child.—M. E. H.

**Renal Adenocarcinoma with Metastasis to the Brain.** Musa, A. B., and Miller, B. [Veterans' Administration, Huntington, W. Va.] *M. Bull. Vet. Admin.*, **17**:309-313. 1940-41.

A case report.—M. E. H.

**Lipo-Fibro-Sarcoma of the Kidney.** Pearse, R. [Toronto Gen. Hosp., Toronto, Canada] *Canad. M. A. J.*, **46**: 127-129. 1942.

A report of 3 cases of a relatively rare type of tumor, only 7 similar ones being found in the literature. The histological picture is that of lipofibromas containing sarcoma cells that are invading the kidney. Gross differences seen in individual cases may be due to differences in rate of growth of the respective benign and malignant elements of the tumor. The paper is illustrated.—A. C.

**Malakoplakia of the Urinary Bladder and Generalized Sarcoidosis, Striking Similarity of Their Pathology, Etiology, Gross Appearance and Methods of Treatment.** Redewill, F. H. [San Francisco, Calif.] *J. Urol.*, **49**:401-407. 1943.

The similarity of malakoplakia of the urinary bladder and Boeck's sarcoid is discussed and illustrated. The main features in common are: (1) large epithelial cells, (2) giant cells, with (3) corpus amylaceous calcium inclusions, (4) absence of symptoms except of mechanical nature, (5) the usually spontaneous healing, (6) an increase in plasma globulin fraction in proportion to the amount of diseased tissue, (7) a tendency to umbilication but not to ulceration and absence of tubercle bacilli in culture, (8) a coincidental tuberculous diathesis. Two cases are described. One patient had both malakoplakia of the bladder and sarcoidosis. Histologically the lesions were the same.—V. F. M.

**Angioma of the Kidney.** Riley, A., and Swann, W. A., Jr. [Boston City Hosp., Boston, Mass.] *Urol. & Cutan. Rev.*, **45**:377-382. 1941.

Angioma of the kidney is a rare disease, the authors finding only 36 acceptable cases recorded in the literature. Hematuria was the cardinal symptom. Nephrectomy is the treatment of choice, especially if large hemorrhages occur. A rather typical case is reported in detail with illustrations.—V. F. M.

**Neurofibroma of the Kidney.** Saphir, O., and Appel, M. [Michael Reese Hosp., Chicago, Ill.] *Urol. & Cutan. Rev.*, **45**:635-639. 1941.

In routine postmortem examinations, small renal tumor

nodules are frequent, and if not adenoma, are usually called fibroma. The authors cite Newcomb who found adenomas in 7% of all autopsies and stated that fibromas were more frequent. The fibromas, or fibroma-like lesions, are usually small grayish nodules found occasionally in the cortex but more often in the medulla. Histologically they bear a close resemblance to benign fibromas. Two thousand consecutive autopsies were reviewed, 806 being on children. No incidental renal tumors were found in the children; the youngest patient with adenoma was 25 years old and the youngest with "fibroma," 35. In the 1,194 autopsies of adults, 68 (5.77%) renal tumors were found, 32 being adenoma and 25 "fibroma," 8 hypernephroid carcinoma, and 3 fibroma with adipose elements. The frequency of both types increased with the age of the patients. The fibromas were studied not only with the routine stains but also with the Van Gieson, Bielschowsky, Masson, and Bodian technics. The presence of fibrils with the morphology and staining characteristics of neurofibrils was thus established in 19 instances. The distribution of the cells in sheaves or whorls, the frequent palisade formations, and the universal nonencapsulation of the tumor with often a strong suggestion of invasion, also support the view that many of these lesions are actually neurofibromas. In several tumors that were much hyalinized, the above points could not be demonstrated. The recent tendency to regard skin fibromas as neurofibromas is a parallel situation. In general no relation of these renal lesions to von Recklinghausen's disease was found. One previously reported neurofibrosarcoma is also described.—V. F. M.

**A Review of Primary Carcinoma of the Ureter.** Scott, W. W. [Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.] *J. Urol.*, **50**:45-64. 1943.

Two cases of primary ureteral carcinoma treated surgically are carefully described and illustrated. A review of the literature found 180 previous reports. The tumors may be divided into the papillary and nonpapillary types with the former much more common. Metastases were found in 62 cases, the regional nodes being most frequently involved. The sixth and seventh decades of life seem most affected. The youngest patient recorded was 22, the oldest 89. The lower third of the ureter is by far the most frequently affected segment. Hematuria and pain are the most usual symptoms. The diagnosis depends on careful complete urological study. In some instances tumor cells have been found in the urine. One hundred and forty-nine patients were operated upon with a postoperative mortality of 24%; 22 died within a year of operation. Those that survive 4 or more years usually die of other diseases or are well. Removal of kidney, ureter, and a cuff of bladder is usually the procedure of choice. Results obtained by preoperative x-radiation are negligible.—V. F. M.

**Goblet-Cell Adenocarcinoma of the Urinary Bladder: Report of Two Cases.** Sumner, W. A., and Gaynor, E. P. [San Francisco, Calif.] *J. Urol.*, **49**:419-425. 1943.

Two instances of this disease are described and illustrated. The etiology is discussed, and particular attention is directed to the possible urachal origin of those tumors occurring in the dome of the bladder.—V. F. M.

**Carcinoma of the Female Urethra.** Tuta, J. A., and Hess, E. F. [Grant Hosp. and Univ. of Illinois Sch. of Med., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **42**:142-143. 1941.

A case report. It is concluded that squamous cell carcinoma of the female urethra, if not recognized very early in the course of the disease, offers a poor prognosis. Metastases occur readily by way of the venous channels in the wall of the urethra.—A. K.

**Cancer of the Urinary Bladder; Analysis of Fifty-One Cases.** Wear, J. B., and Kohler, H. H. [Madison, Wis.] *Wisconsin M. J.*, **41**:392-394. 1942.

The type and grade of the cancer, the presence of renal complications, the choice of treatment, and therapeutic results are discussed.—M. E. H.

## Oral Cavity and Upper Respiratory Tract

**Common Lesions of the Larynx.** Bumgardner, J. S. [Louisville, Ky.] *Kentucky M. J.*, **40**:22-24. 1942.

A short discussion of common lesions of the larynx is presented with a description of the routine procedure for their systematic study.—J. L. M.

**Adamantinoma of the Floor of the Right Nostril. Occurrence in a Child Aged Five Years.** Berkove, A. B. [Children's Hosp. of East Bay, Oakland, Calif.] *Arch. Otolaryng.*, **35**:928-931. 1942.

A case report. The tumor was characterized by its slow growth and its recurrence after high voltage roentgen irradiation.—M. E. H.

**Radio-Resistant Cancer of the Upper Lip: A Case Report.** Bradley, E. T. [New Canaan, Conn.] *Connecticut M. J.*, **7**:166-168. 1943.

Radical surgery followed by plastic repair was resorted to when treatment with radium was unsuccessful on 2 occasions.—M. E. H.

**Bowen's Disease and Superficial Mucosal Epithelioma of Upper Respiratory and Alimentary Tracts.** Brighton, G. R., and Altman, F. [Coll. of Physicians and Surgeons, New York, N. Y.] *Arch. Otolaryng.*, **36**:354-371. 1942.

Two typical cases of Bowen's disease of the mucous membrane are reported. The relations between Bowen's disease and bowenoid epithelioma of the mucous membrane are discussed. Histologically the two lesions are closely related and belong to the same group of changes.—M. E. H.

**Hoarseness and Cancer of the Larynx.** Clerf, L. H. [Philadelphia, Pa.] *West. J. Surg.*, **49**:599-602. 1941.

The importance of hoarseness as the most frequent and earliest symptom of laryngeal cancer involving a vocal cord is stressed.—M. E. H.

**Cancer of the Lips and Cheeks.** Figi, F. A. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:73-75. 1941.

A general discussion.—J. L. M.

**Tumours of the Larynx.** Fletcher, G. W. [Winnipeg, Canada] *Canad. M. A. J.*, **46**:474-477. 1942.

A survey of the methods in use for the treatment of both benign and malignant growths of the larynx.—A. C.

**Ganglion Cell Neuroma of the Pharynx.** Foster, J. H. [Houston, Tex.] *Arch. Otolaryng.*, **36**:372-376. 1942.

A case report. A review of the literature would indicate that nerve tissue tumors of the pharynx are uncommon.—M. E. H.

**Chondroma of the Larynx. Report of Two Cases in Which Laryngofissure Was Employed.** Gatewood, E. T. [Med. Coll. of Virginia, Richmond, Va.] *Arch. Otolaryng.*, **36**:187-197. 1942.

From a review of the recorded cases, it seems to the author that a large percentage of chondromas of the larynx are not encountered by the laryngologist until tracheotomy is urgently needed or until the growth has advanced to a stage at which total laryngectomy is required.—M. E. H.

**Cancer of the Larynx.** Gill, E. G. [Roanoke, Va.] *Virginia M. Monthly*, **68**:509-512. 1941.

Recommendations for diagnosis, grading, and treatment are presented.—M. E. H.

**Diagnosis and Treatment of Cancer of the Larynx. With a Statistical Review of Fifteen Cases.** Hammond, A. E. [Detroit, Mich.] *Arch. Otolaryng.*, **35**:1-29. 1942.

A study of 15 patients with carcinoma of the larynx revealed that the highest incidence was in the 40 to 70 year age group with males predominating. Vocal abuse and the use of tobacco ranked high as contributing causes. Eighty per cent of the cancers were intrinsic, 6.6 extrinsic, and 13.2 mixed. Hoarseness was the most prominent symptom. Biopsy was the one indisputable diagnostic procedure in all the cases. Eighty per cent of the cancers were of the well differentiated types of tissues of grade 1 or 2, the other 20% were of the anaplastic group, grades 3 and 4. Surgical intervention, either by laryngofissure or by laryngectomy, is the procedure of choice in the treatment of intrinsic carcinoma of the larynx; irradiation in the treatment of extrinsic carcinoma.—M. E. H.

**Cancer of the Tongue.** Havens, F. Z. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:72-73. 1941.

Malignant tumors involving the tongue and floor of the mouth are more difficult to treat successfully than growths in other locations of the mouth because such tumors usually are highly malignant and because the extreme vascularity of the tongue and its rich lymphatic supply greatly increase the likelihood of early metastasis.

The malignant tumor encountered most frequently in the tongue is squamous cell carcinoma, but occasionally hemangioendotheliomas, adenocarcinomas, and lymphosarcomas are seen. Patients with malignant lesions of the tongue are males in 90% of cases and the majority of these are between 45 and 60 years of age. In the differential diagnosis of carcinoma of the tongue, gumma, thickened leukoplakia, benign papillary lesions, infected cysts, fibromas, tuberculosis, actinomycosis, and blastomycosis must be excluded. Acutely inflamed angiomas, especially lymphangiomas, may be mistaken for carcinoma. At the junction of the margin of the tongue and the anterior tonsillar pillar there is a normal accumulation of lymphoid tissue. In an occasional patient this lymphoid tissue may be prominent and may be mistaken for carcinoma. Biopsy of suspected lesions should be carried out if the diagnosis cannot be established otherwise.

Surgical removal of the regional lymph nodes should be carried out in all cases in which the local lesion permits of this procedure. If the tumor is active in type, postoperative irradiation is advisable. If, for any reason, surgical removal of the nodes is not carried out, treatment by irradiation alone is indicated. In a group of 162 patients treated at the Clinic according to the plan outlined above, it has been possible to trace 156, and of these 37.2% were found to be living and well 5 or more years after treatment.—J. L. M.

**Papilloma of the Tonsil and Uvula.** Henner, R. [Chicago, Ill.] *Arch. Otolaryng.*, **35**:810-811. 1942.

The author regards papillomas of the tonsils, tonsillar pillars, and uvula as common lesions, since their occurrence was 1 in 266 among 25,000 routine ear, nose, and throat examinations.—M. E. H.

**Chondroma of the Larynx.** Holinger, P. H., and Matzkin, L. L. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Arch. Otolaryng.*, **35**:640-645. 1942.

A case report that is of interest because of complete restoration of function following the relatively early removal of the tumor.—M. E. H.

**Carcinoma of the Larynx in a Girl Aged 15.** Jones, A. C. [Boise, Idaho] *Arch. Otolaryng.*, **35**:426-430. 1942.

A case report with autopsy findings. The conclusion is reached that any child with hoarseness should receive a very careful laryngeal examination, because it is now demonstrated that such a child may have carcinoma of the larynx.—M. E. H.

**Laryngeal Carcinoma.** McCall, J. W., and Lamb, A. J. [St. Luke's Hosp., Cleveland, Ohio] *Ohio State M. J.*, **37**:1053-1055. 1941.

Nineteen patients with this tumor are listed, and symptoms, age, pathologic findings, operation, and result are recorded. Fifteen total laryngectomies were performed without operative mortality. All patients with intrinsic tumors are living and well; 4 of the 10 with tumors that had progressed beyond the laryngeal wall were also successfully treated.—E. E. S.

**Tumors of the Palate (Benign and Malignant).** Martin, H. [Memorial Hosp., New York, N. Y.] *Arch. Surg.*, **44**:599-635. 1942.

All cases of oral cancer admitted to Memorial Hospital between 1929 and 1936 inclusive were analyzed, and 8% of the lesions were classified as cancer of the palate. In the latter group the ratio of malignant to benign growths was 12 to 1. The average age of persons in this series was 57 years. The mean age of those with epidermoid tumor was 60 years; with adenocarcinoma, 48 years; and with mixed tumor, 31 years. Of the patients with epidermoid carcinoma, 90% were males and 19% females. The palatal adenoid tumors showed a higher incidence in females (58%) than in males (42%). Collectively, these neoplasms occurred more often in the soft than in the hard palate, although the incidence of tumors of the salivary gland type at the 2 sites was 43 and 57% re-

spectively. Leukoplakia, chronic glossitis, and atrophy of oral mucous membranes were considered important etiological factors for the epidermoid tumors. In the adenoid type the mucous membranes showed no evidence of chronic irritation. Metastasis occurred in 61% of the patients during the course of the disease, tumors of the soft palate being much more likely to metastasize than those of the hard palate. Three patients died with systemic metastases. Histologically, 7% of these tumors were benign; 83% were epidermoid carcinoma; the remainder were adenocarcinoma except for one melanoma and one rhabdomyosarcoma. Five year survivals occurred in 23% of the cases. Methods of treatment were discussed.—R. C. R.

**Cancer of the Lip.** Martin, H., MacComb, W. S., and Blady, J. V. [Memorial Hosp., New York, N. Y.] *Ann. Surg.*, **114**:226-242 and 341-368. 1941.

An intensive analysis of 375 consecutive cases, making up 4% of all admissions for cancer at the Memorial Hospital for a 7 year period. The lesion is defined as strictly limited to the mucous membrane (93% incidence on the lower lip), and is considered one of the least malignant forms of intraoral cancer. Cervical node metastases occur late and usually in orderly fashion. The uncomplicated primary lesion may be treated by surgery or radiation with equally good chance of cure. Surgical and radiation technics are thoroughly discussed for all types of cases. The net 5 year cure rate in this series is 70%.—M. R. D.

**Carcinoma of the Maxillary Antrum Treated by Radium and Surgery.** Mill, W. A., and Lederman, M. *Proc. Roy. Soc. Med.*, **36**:358. 1943.

Description of a case.—E. L. K.

**Suspension Laryngoscopy in the Treatment of Malignant Disease of the Hypopharynx and Larynx.** New, G. B., and Dorton, H. E. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:411-416. 1941.

Although suspension laryngoscopy has been used for many years, its value in the treatment of malignant diseases of the hypopharynx and larynx is not generally recognized. The authors feel that it has a definite place in the treatment of these lesions because of the lessened surgical risk for carefully selected patients.

During the period from 1927 to 1937 inclusive, of 450 patients with malignant disease of the hypopharynx and larynx seen at the Mayo Clinic, a selected group of 38 were treated with the aid of suspension laryngoscopy. Surgical diathermy and radium emanation seeds have been employed, and in addition external irradiation has been used at times. Immediate histological diagnosis of the freshly frozen section is an essential part of this method. The authors report the mode of selection of their 38 cases, the method of treatment, and its results.—J. L. M.

**Tumors of the Nose and Throat.** New, G. B., and Erich, J. B. [Mayo Clinic, Rochester, Minn.] *Arch. Otolaryng.*, **36**:566-589. 1942.

Under the heading of Progress in Otolaryngology the author abstracts articles appearing in the more recent literature. Emphasis is placed on methods of treatment.—M. E. H.

**An Unusual Polypoid Tumor of the Nasal Choana.** Pastore, P. N., and Lillie, H. I. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:730-732. 1941.

Solitary polyps in the nasal choana occur relatively infrequently. They usually rise in the maxillary sinus and frequently can be traced as far as the ostium of the maxillary sinus where a pedicled stalk may be seen. They also may arise from other nasal sinuses and from the mucosa of the nasal chamber, particularly that of the middle and superior turbinates.

Choanal polyps are commonly of the fibromyxomatous type and present a smooth surface. Other polyps may or may not be present in the nasal chamber at the same time.

The present report is made because of the rarity of lesions of the type described and because examination of the specimen removed at operation led to the conclusion that the lesion may have been present for a much longer period than the patient had been aware of.—J. L. M.

**El tratamiento del cáncer de la laringe en el Instituto de medicina experimental. [Treatment of Cancer of the Larynx in the Institute of Experimental Medicine.]** Roffo, A. H., and Ferrari, R. C. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **18**:1083-1126. 1941.

The methods followed are thus summarized: (1) Radical surgery. Removal of the larynx, which is done in all cases of intralaryngeal tumors, or in those in which, despite a moderate invasion of adjacent tissues, the growth still can be removed. (2) Roentgentherapy. Used in cases in which surgical treatment is impossible either because of the poor general condition of the patient or the extensive invasion of local tissues. (3) Palliative treatment. Applied to hopeless cases.

The technic of removal of the larynx is described in detail and is illustrated by 18 figures.—M. D-R.

**Transseptal Removal of Chondroma Replacing Sphenoid Sinus and Sella.** Soper, G. R., and Galloway, T. C. [Evanston, Ill.] *Arch. Otolaryng.*, **36**:126-130. 1942.

A case report. The comparative rarity of endochondromas of the sphenoid sinus and the ease of removal by rhinologic approach make the case of interest.—M. E. H.

**Hoarseness, an Important Symptom.** Watkins, S. [Louisville, Ky.] *Kentucky M. J.*, **39**:134-138. 1941.

In describing conditions in which hoarseness occurs, the author discusses the following neoplasms: postcricoid carcinoma, singer's nodes on the vocal cords (bilateral edematous fibromas), and papilloma, epithelioma, and sarcoma of the larynx.—J. L. M.

**Malignant Tumors of the Accessory Nasal Sinuses.** Weinberger, N. S. [Robert Packer Hosp., Sayre, Pa.] *Pennsylvania M. J.*, **45**:719-723. 1942.

The important factors governing the therapeutic management of malignant tumors of the accessory nasal sinuses are: (1) location of the tumor, (2) extent of involvement, (3) presence or absence of metastasis, (4) grade of malignancy, (5) size, (6) accessibility, (7) age and condi-

tion of the patient. Irradiation, particularly with radium, is now more effectively used than formerly and is preferred in the higher grade malignant growths. In cases where diffusion of irradiation is required, deep roentgen therapy is preferable, either alone or, better still, in conjunction with radium. Malignant growths of the inferior section are best treated with surgical diathermy or excision, plus radium. Early diagnosis and intensive treatment are highly desirable. All tissue removed from the nose and sinuses should be subjected to microscopic study. Reports of 2 cases are presented.—J. L. M.

**Cancer of the Lip.** Editorial. *Rocky Mountain M. J.*, **39**:413. 1942.

Although treatment of lip cancer is a moot question, to the author the best procedure seems to be clear cut in many cases: Irradiation for early and small lesions; conservative local surgery for larger lesions without palpable neck glands; radical surgery with neck dissection when the cervical region is involved.—M. E. H.

## Gastrointestinal Tract

**Early Symptoms of Carcinoma in the Colon and Rectum.** Abell, I., Jr. [Louisville, Ky.] *Kentucky M. J.*, **40**:219-223. 1942.

The signs, symptoms, and diagnostic procedures in cases of early carcinoma in the colon and rectum are discussed. —J. L. M.

**Carcinoma of the Esophagus.** Adams, H. D. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North America*, **22**:709-719. 1942.

Resection of the esophagus at any level for carcinoma is a fairly well standardized procedure. The methods of resection of the cervical, thoracic upper two-thirds, and thoracic lower third are described in detail. Early diagnosis and operation can save increasing numbers of patients with carcinoma at any level of the esophagus.—J. L. M.

**Important Newer Concepts concerning Cancer of the Intestines and Their Bearing on Management.** Bargen, J. A. [Mayo Foundation, Rochester, Minn.] *West Virginia M. J.*, **37**:433-442. 1941.

The author views the mucosal cells of the intestine as reflecting a constant antagonism between cells multiplying in a purposeful, orderly, fashion and those wholly useless, which are essentially parasitic. The carcinoma represents the ultimate phase of a series of changes whereby the latter group attain ascendency. The existence of potentially malignant states has been demonstrated clinically by the recurrence of benign tumors surgically removed, and pathologically by the appearance of numerous local areas of hyperplasia. Enlarged lymph follicles disrupting the muscularis and mucosa, and forming ulcers, or covered by more primitive appearing epithelium, may be contributing factors in the etiology of malignant disease of the bowel. Single polyps are regarded as potential carcinomas, and their removal is advised. In multiple polypoid or adenomatous disease of the colon, frank carcinoma was found in 62.5%. In 25% of these, the carcinoma was also multiple. Familial incidence was determined in 35%. In a study of 176 patients with cancer of the colon, familial incidence of cancer, usually in the colon or stomach, was found to be twice as frequent as in a control group of similar size. When one malignant colon tumor is removed, a new growth may appear owing to the potentially malignant state of the mucosa in many areas. Adenomas and eventually malignant tumors have been seen as a sequel to chronic ulcerative colitis with its disorderly process of healing. All minor lesions should be studied carefully by surgeon and pathologist for evidence of malignancy, and frequent check-ups for several years after removal of benign lesions are important. A diet rich in carbohydrate with minimal residue, warm saline irrigations, and intraperitoneal vaccine given postoperatively are advised to minimize infection.—E. E. S.

**Carcinoma of the Large Bowel, 1931 to 1938 Inclusive, St. Mary's Hospital, Waterbury.** Burke, J. F. [Waterbury, Conn.] *Connecticut M. J.*, **6**:364-365. 1942.

Of 103 patients seen between 1931 and 1938 at this hospital, 44.6% were operated upon, 26.2% having radical procedures. Three year survivals were obtained in 30.4% and 5 year survivals in 15.2% of the patients operated upon. Poor survival rates and the low percentage of operation were attributed to the advanced stage of the disease when the patients were first seen.—R. C. R.

**Carcinoma of Large Intestine.** Butler, P. F. [Boston, Mass.] *Connecticut M. J.*, **6**:359-361. 1942.

The clinical and pathological characteristics of the lesions in the large bowel are considered from the diagnostic point of view with emphasis on roentgenographic and proctologic examination. Differential diagnosis is discussed.—R. C. R.

**Selection of Operation for Cancer of the Colon and Rectum.** Cattell, R. B. [Boston, Mass.] *Connecticut M. J.*, **6**:361-364. 1942.

All primary carcinomas of the large bowel should be resected whenever it is technically feasible unless massive liver metastases are present. Patients should be thoroughly studied and prepared preoperatively in order that as extensive an operation as may be found necessary can be done. Types of surgical procedure most suitable to the individual case must be chosen.—R. C. R.

**Regional Lymphatic Metastases of Carcinoma of the Stomach.** Coller, F. A., Kay, E. B., and McIntyre, R. S. [Univ. of Michigan, Ann Arbor, Mich.] *Arch. Surg.*, **43**: 748-761. 1941.

An analysis of the incidence of metastases in regional lymph nodes in 53 cases of carcinoma of the stomach is presented. Evidence of metastasis was found in 75.5% of the cases. There was no relation between either duration of symptoms or size of neoplasm and incidence of metastases. Ninety-five and four tenths per cent of the sessile tumors and 60% of the polypoid tumors had metastasized. The lymph nodes most frequently involved were the inferior gastric–subpyloric group and the superior gastric group. In 26.4% of the cases the carcinoma had involved the duodenum, and in 24.5% the upper margin of the tumor could not be determined by palpation alone at the time of operation. The authors recommend wide resection of the stomach including the 4 zones of regional lymph nodes. —G. H. H.

**Sarcoma of the Small Intestine.** Frank, L. W., Miller, A. V., and Bell, J. C. [Univ. of Louisville Sch. of Med., Louisville, Ky.] *Ann. Surg.*, **115**:544-565. 1942.

The authors present a critical analysis and comprehensive discussion of the primary sarcomas of the small intestine reported in the past 10 years. Four case studies are added.—M. R. D.

**Carcinoma of the Rectum.** Gilchrist, R. K. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Surg. Clin. North America*, **23**:221-245. 1943.

A review of carcinoma of the rectum including the following subjects: (1) symptoms: change in bowel habits, passage of blood, backache; (2) differential diagnosis: hemorrhoids, polyps, diverticula; (3) diagnostic procedures: rectal, x-ray, and proctoscopic examination; (4) prognosis: based on size of tumor, enlargement of the lymph nodes, fixation of the tumor, age of patient; (5) preoperative preparation; (6) treatment of complete or almost complete obstruction: cecostomy, transverse colon colostomy, sigmoid colostomy; (7) resection of the colon for carcinoma: sigmoid carcinoma, (8) abdominoperineal resection for carcinoma of the rectum: detailed technic of operative procedures, description of postoperative care, and complications; and (9) inoperable carcinomas.—J. L. M.

**Ulcer and Cancer of the Stomach in the Middle Ages. Avenzoar, Averroës, Franciscus (of Piedmont), Benivieni.** Goldstein, H. I. [Camden, N. J.] *Rev. Gastroenterol.*, **10**:157-162. 1943.

Historical review showing how old modern medicine is.—M. E. H.

**Pernicious Anemia and Cancer of the Stomach.** Hagyard, C. E. [Seattle, Wash.] *Northwest Med.*, **40**:125-127. 1941.

The case reported is that of a man who had a partial gastrectomy for an obstructing pyloric carcinoma at the age of 30. Subsequent gastritis resulted in complete absence of hydrochloric acid and ferments. Pernicious anemia developed 9 years after operation. Another carcinoma in the fundus of the stomach and lower esophagus appeared 6 years later.—E. E. S.

**Carcinoma of the Stomach Emphasizing Some of the Misconceptions of the Disease.** Hinton, J. W. [New York Post-Graduate Med. Sch., New York, N. Y.] *Bull. New York Acad. Med.*, **17**:829-843. 1941.

According to the Metropolitan Life Insurance Company statistics (1911–1935), this tumor comprises 20.4% of the carcinomas. Of the total deaths, 1.8% are due to stomach carcinoma. The author believes few carcinomas develop on the basis of a benign ulcer. The gastrointestinal x-ray series is regarded as the best means of differentiation, and low free hydrochloric acid should lead to suspicion of the presence of a tumor despite negative x-ray findings. In the series of patients reported, a short duration of symptoms did not improve chances of operative cure. A brief discussion of the role of known carcinogenic agents and of the glands of internal secretion in production of gastric carcinoma is appended. It is thought that the secretions of the gastric mucosa are affected by alteration in endocrine activity.—E. E. S.

**Some Aspects of Cancer of the Stomach.** Horsley, J. S. [St. Elizabeth's Hosp., Richmond, Va.] *Virginia M. Monthly*, **70**:18-24. 1943.

Trivial symptoms of indigestion in persons over 30 years of age, especially in men, should be considered seriously and the cause of the symptoms ascertained. Diagnostic aids and treatment are reviewed.—M. E. H.

**A Modified Mikulicz Resection for Cancer of the Colon.** Lahey, F. H., and Colcock, B. P. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North America*, **22**:773-782. 1942.

The authors believe that some type of exteriorization operation remains the safest method for resection of cancer of the colon, even in the hands of the experienced surgeon. Peritonitis, which is still the most frequent cause of death following a one stage resection with primary anastomosis, occurs infrequently after the exteriorization operation and then is usually secondary to complications such as a perforation of the growth or a severe wound infection. The technic of the modified operation is described in detail.—J. L. M.

**Hodgkin's Disease of the Duodenum.** Pusch, L. C. [York Hosp., York, Pa.] *Pennsylvania M. J.*, **45**:20-21. 1941.

The literature on Hodgkin's disease of the gastrointestinal tract is briefly reviewed. The case reported here resembles those instances of Hodgkin's disease of the stomach or intestines in which the lesion is a mucosal ulceration bordered by indurated tissue having the characteristic histopathology of lymphogranulomatosis. It is unusual in that it resulted in a wide perforation of the anterior duodenal wall just distal to the pylorus, with the development of a peripyloric peritoneal abscess of nonspecific type.

Although Hodgkin's disease may be primarily in the wall of the stomach or intestine, arising from the intramural lymphoid follicles, in the present case it seems likely that the duodenal involvement is an extension from regional lymph nodes. This is suggested by the greater involvement of duodenal and gastric serosa and gastrohepatic ligament, and the wedge-shaped extension into the duodenal wall, the apex of the wedge reaching the mucosa at the site of perforation.—J. L. M.

**Diagnostic Points in Rectal Cancer.** Rosser, C. [Baylor Univ., Dallas, Tex.] *J. Oklahoma M. A.*, **34**:1-4. 1941.

It is emphasized that many malignant epithelial tumors of the rectum and rectosigmoid junction arise in adenomas or inflammatory conditions. Sixty per cent of patients with malignant tumors situated near the anal canal complained of pain as the initial symptom. When the carcinoma was located in the rectosigmoid junction, constipation was common as an early complaint, and bloody diarrhea was present at some time in the majority of patients. The cardinal point of the physical examination is that rectal carcinoma is a single indurated ulcerated lesion surrounded by normal mucous membrane with an abrupt line of demarcation.—E. E. S.

**Total Gastrectomy for Carcinoma. Report of a Successful Case.** Saint, J. H. [Sansum Clinic, Santa Barbara, Calif.] *West. J. Surg.*, **51**:10-16. 1943.

A case report. Scirrhous carcinoma involved nearly all the stomach except the pyloric end. Removal of the

stomach was followed by esophagojejunostomy. Recovery was prompt, and convalescence uneventful; the patient lived 14 months. The operation consisted of total gastrectomy, antecolic esophagojejunostomy, and jejunojejunostomy. Death was due to metastases.—M. E. H.

**Superficial Spreading Type of Carcinoma of the Stomach.** Stout, A. P. [Coll. of Physicians and Surgeons, New York, N. Y.] *Arch. Surg.*, **44**:651-657. 1942.

In a series of 15 cases, it has been possible to demonstrate a type of carcinoma of the stomach that originated in the mucosa and submucosa and spread peripherally in these areas to attain a rather large size before involving the muscularis of the gastric wall. In all these cases the antral portion of the stomach was involved. In the gross the carcinoma was red, irregular, and slightly nodular with a shallow bowl-shaped or deep penetrating ulcer. Despite the superficial location of these tumors, 7 of the 15 under discussion had already metastasized before operation. Most of these patients had a history of symptoms persisting from 1 to 10 years. Nearly 22% of the gastric carcinomas resected at the Presbyterian Hospital between 1937 and 1941 showed this type of lesion. Histologically the lesion was variable but always showed relatively superficial growth and extension.—R. C. R.

**Cancer of the Stomach.** Walker, R. H. [Charleston, W. Va.] *West Virginia M. J.*, **37**:345-348. 1941.

A general discussion including symptoms such as anorexia, epigastric distress, nausea and vomiting, loss of weight, general debility, and hemorrhage. Gastric acidity is often low. X-ray examination is highly efficient in establishing diagnosis. Surgical removal of the tumor is urged if only for temporary improvement; palliation alone is regarded as ample excuse for operation.—E. E. S.

**Gastric Ulcer, Carcinomatous Ulcer or Ulcerating Carcinoma?** Walters, W. [Mayo Clinic, Rochester, Minn.] *Ann. Surg.*, **115**:521-529. 1942.

The treatment of gastric ulcers should depend on the pathologic nature of the lesion. A definite incidence, 10 to 20%, of carcinomatous ulcers cannot be distinguished by any special symptom complex, roentgenographically, or by their response to a medical regimen. The most effective surgical procedures for specific entities are discussed.—M. R. D.

**Malignant Lesions of the Stomach.** Walters, W. [Mayo Clinic, Rochester, Minn.] *Arch. Surg.*, **44**:636-650. 1942.

Cancer of the stomach affects males 3½ times more frequently than it does females. Of the patients reported here, 45% of those who at gastric resection had no extension of the lesion were alive after 5 or more years. Of the patients treated surgically, 31.9% survived at least 5 years irrespective of the grade of malignancy or degree of involvement of the regional lymph nodes at the time of operation. The more malignant the lesion as determined by Broder's index, the smaller was the percentage of 5 year survivals. In females, the resectability was higher and operative risk lower than it was in males. Of the original group, 1.7% had sarcoma. There was a high incidence of ulcer-like dyspepsia in the patients reported. In 80.6% of those with dyspepsia, the symptom was relieved by a nonsurgical regimen, thus clearly showing the importance of a thorough and careful diagnostic procedure. It was also shown that the roentgenologist was able to make a positive diagnosis of carcinoma in only 75.3% of the cases in which he could demonstrate a gastric lesion. The free hydrochloric acid content of 20% of the patients in this series was more than 30 degrees. The 1940 mortality rate for patients treated by surgery was 8.5%, while the rate for the entire series was 16%. Precancerous lesions of the stomach should be removed surgically whenever found.—R. C. R.

**Primary, Solitary Lymphoid Tumors of the Gastro-Intestinal Tract.** Warren, S., and Lulenski, C. R. [Harvard Med. Sch., Boston, Mass.] *Ann. Surg.*, **115**:1-12. 1942.

Solitary, lymphoid tumors of the gastrointestinal tract are of not infrequent occurrence and should be considered in a differential diagnosis. In a series of 3,132 malignant lesions, 28 were of the solitary, lymphoid type, an incidence of 0.9%. A comprehensive discussion of the clinical and pathologic features is presented.—M. R. D.

**Leiomyoma—A Tumor with Typical Roentgenological Appearance.** Editorial. *Rev. Gastroenterol.*, **10**: 132-133. 1943.

The outstanding gross anatomical features of these tumors are that they are sharply circumscribed, round, and mostly single. The roentgenographic picture is characteristic, showing a large, round, punched-out area in the middle of the stomach.—M. E. H.

## Liver

**Fibroma del hígado-exeresis-curación. [Successful Removal of a Fibroma of the Liver.]** Chifflet, A. [Montevideo, Uruguay] *Arch. urug. de med., cir. y especialid.*, **20**:44-53. 1942.

Description of a case in which surgical removal of a fibroma of the liver was successfully carried out. The tumor measured 20 cm. in diameter, and, according to the author, is the largest of the kind ever reported as having been operated upon. The surgical procedure followed is fully described, with some general considerations on the disease. Two illustrations are appended.—M. D-R.

**Primary Carcinoma of the Liver.** Upham, R., and Klotz, S. D. [New York Med. Coll., New York, N. Y.] *M. Clin. North America*, **26**:855-863. 1942.

The high incidence of primary carcinoma of the liver in the male sex and in the period between 40 and 60 years of age, the usual cirrhotic background, and the greater frequency in the dark skinned races are noted. The clinical features of rapid onset, marked asthenia, short duration, otherwise unexplainable mild fever, ascites often sanguineous, constant dull pain in the right hypochondrium, an enlarging tender and nodular liver, leukocytosis, and the infrequent evidence of metastasis are discussed. The finding of an increasing alkaline serum phosphatase without other laboratory evidence of obstructive disease in the liver is stressed and regarded as strongly suggestive of carcinoma of this organ.

The utilization of peritoneoscopy in diagnosis is advised. The differential diagnosis of primary carcinoma of the liver is discussed. That it may sometimes present the picture of an acute surgical abdomen is to be remembered. Surgical excision of the tumor whenever possible is the accepted treatment at present.—J. L. M.

**Some Aspects of the Pathology of Carcinomata of the Biliary Tract.** Willis, R. A. [Alfred Hosp. and Univ. of Melbourne, Melbourne, Australia] *Med. J. Australia*, **2**:340-344. 1942.

A comparison is made of the findings in cases of carcinoma of the bile ducts with those in examples of carcinoma of the gall bladder and of the pancreas, seen postmortem during the last 5 years. The material is classified as follows: carcinoma of the main bile ducts (15 cases), gall bladder and bile ducts (3 cases), gall bladder (11 cases), and pancreas (14 cases). The series suggests that bile duct carcinoma is at least as common as carcinoma of the gall bladder and of the pancreas. Gall bladder carcinoma was more frequent in the women of the series, but this did not hold for carcinoma of the ducts. Of the 11 gall bladder tumors, 10 were associated with gallstones, while in none of the 15 cases of bile duct carcinoma were stones present in the affected ducts. The extent of distribution of many of these tumors of the biliary system pointed to a diffuse or multicentric origin, and multiple origin was clearly shown in one case of carcinoma of the gall bladder with 2 distinctly separate areas of growth, each related to a stone. It is suggested that experiments on the induction of cancer by gallstones (so far quite negative) might be repeated with stones from cancerous human gall bladders, and that such stones might be examined in other ways for the presence of carcinogenic substances possibly derived from the bile acids.—Adapted from A. H.

## Bone and Bone Marrow

**Comentarios sobre tres autopsias de sarcomas de Ewing. [Comments on Three Autopsies on Ewing's Sarcoma.]** Bianchi, A., and Muscolo, D. [Buenos Aires Sch. of Med., Buenos Aires, Argentina] *Rev. ortop. y traumatol.*, **12**:3-16. 1942.

Three cases of Ewing's sarcoma are reported.—M. D-R.

**Cancer of the Jaws.** Erich, J. B. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:75-77. 1941.

In addition to modern methods of therapy, much of the success in the management of patients with cancer of the jaws is the result of the visibility and accessibility of the growths, the low degree of malignancy, and the delayed tendency of such tumors to give rise to metastases. Malignant tumors of the jaws may be classified as squamous cell epitheliomas, various forms of sarcomas, adenocarcinomas of the mixed tumor type, and adamantinomas. Although a few of the epitheliomas and sarcomas are highly active, fulminating growths, the majority of these tumors are rather low grade in character. Each of these classes is described and the recommended therapy discussed.—J. L. M.

**Giant Cell Tumor with Malignant Change.** Hazel, O. G., Chont, L. K., and Hull, W. M. [Oklahoma City, Okla.] *J. Oklahoma M. A.*, **34**:246-248. 1941.

The patient described developed a malignant osteogenic sarcoma 16 years subsequent to a benign giant cell tumor in the lower portion of the right femur. Metastases grew in the neck and lung.—E. E. S.

**Disarticulation of the Hip for Endothelioma (Ewing's Tumor): 31-Year Follow-Up.** Smith, B. C. [New York, N. Y.] *Ann. Surg.*, **115**:318-320. 1942.

A case report emphasizing an unusual interval of survival.—M. R. D.

**Neoplasms of the Bony Thoracic Wall.** Sommer, G. N. J., Jr., and Major, R. C. [Univ. of Michigan, Ann Arbor, Mich.] *Ann. Surg.*, **115**:51-83. 1942.

An analysis of 66 cases of neoplasms of the bony thoracic wall collected from the literature since 1933. Fifteen cases observed at the University of Michigan Hospital are added. The primary interest is concerned with treatment, complete surgical removal being the procedure of choice.—M. R. D.

**Primary Reticulum Cell Sarcoma of Bone.** Szutu, C., and Hsieh, C. K. [Peiping Union Med. Coll., Peiping, China] *Ann. Surg.*, **115**:280-291. 1942.

A detailed report of 2 cases of primary reticulum cell sarcoma of bone with bone regeneration following roentgenotherapy.—M. R. D.

**Rehabilitation after Radical Local Resection of Bone Tumors.** Tuell, J. I. [Seattle, Wash.] *Northwest Med.*, **40**:138-140. 1941.

A rocker bottom shoe proved very successful in restoring normal use of a boy's foot from which a malignant chondroma had been resected. Fusion of the tibia and os calcis had occurred. The second patient described had complete resection of a length of the humerus for a bony proliferative tumor, with replacement of the segment by implantation of a portion of the fibula. She had no disability in the leg and fair function was restored to the arm.—E. E. S.

## Muscle and Tendon

**Enorme lipoma intermuscular del muslo. [Very Large Intermuscular Lipoma of the Thigh.]** Arenas, N. A. R. [Buenos Aires, Argentina] *Publ. méd., São Paulo*, **6**:5-9. 1940.

A report of a case.—M. D-R.

**Synovial Sarcoma with Report of Two Cases.** Lazarus, J. A., and Marks, M. S. [New York, N. Y.] *Surgery*, **13**:290-308. 1943.

A review of the literature and the presentation of 2 cases. Of the 76 recorded cases trauma occurred in 25%. The knee joint was involved in 48.7% of cases, the elbow in 10.5%, the ankle in 9.2%, and the palm of the hand in 4%. In 56.6% prompt recurrence and metastases followed treatment; only 16% of 64 patients survived 5 years. Irradiation is advocated only as an adjuvant to surgical intervention.—W. A. B.

## Blood Vessels

**Treatment of Hemangiomas.** Kinney, K. K. [Willimantic, Conn.] *Connecticut M. J.*, **7**:139-140. 1943.

Of 22 patients treated by 1 of 3 surgical methods; namely, sclerosing solutions, electrocoagulation, or excision, 16 were cured, 3 improved, 1 had a recurrence, and 2 died. Of 74 patients treated by irradiation, including radium, 37 were cured, 33 showed satisfactory results, and 4 failed to respond to treatment.—M. E. H.

## Leukemia, Lymphosarcoma, Hodgkin's Disease

**Lymphatic Leukemia.** Beard, M. F. [Louisville, Ky.] *Kentucky M. J.*, **39**:138-143. 1941.

The physiology of lymphopoiesis is briefly discussed. A classification of lymphatic leukemia into workable clinical groups is attempted.—J. L. M.

**Hodgkin's Disease. A Pathologic Classification.** Bersack, S. R. [Veterans' Administration, Hines, Ill.] *Am. J. Clin. Path.*, **13**:253-259. 1943.

A review of the microscopic slides of 186 cases, including 67 necropsies, with classification into 3 groups: (1) Hodgkin's lymphoreticuloma; (2) Hodgkin's granuloma; and (3) Hodgkin's lymphoma.—J. G. K.

**Acute Lymphatic Leukemia.** Brown, E. H. [Thomas-Davis Clinic, Tuscon, Ariz.] *Southwestern Med.*, **26**:82-84. 1942.

Two cases of acute lymphatic leukemia are reported. Severe hemorrhage following trauma seemed to have been the predisposing factor in these cases.—J. L. M.

**The Lymphoblastomas.** Goldhamer, S. M. [Ann Arbor, Mich.] *Proc. Inst. Med. Chicago*, **14**:190-191. 1942.

The author believes the more inclusive term hemocytoblastoma should be used for this group of diseases since they are not limited to the lymphatic tissue but include all the hematopoietic tissues. A summary of the evidence for considering them to be malignant is reviewed with brief attention to the methods of treatment in use at present.—M. E. H.

**Pregnancy Complicating Hodgkin's Disease (Lymphogranuloma Malignum).** Kushner, J. I. [Bronx Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **42**:536-538. 1941.

A case report of Hodgkin's disease complicated by pregnancy. The patient received radiation therapy during the early months of pregnancy. A healthy child was born whose subsequent development was normal.—A. K.

**Cutaneous Ulcerative Hodgkin's Disease.** Kierland, R. R., and Montgomery, H. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **16**:124-128. 1941.

The authors report a case of Hodgkin's disease that included ulcerating cutaneous nodules with specific and diagnostic histological features and comment briefly on the frequency and character of the cutaneous manifestations in association with Hodgkin's disease.—J. L. M.

**Pregnancy Complicated by Hodgkin's Disease.** Klawans, A. H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **43**:895-896. 1942.

In this case a clinical diagnosis of Hodgkin's disease was made during the second trimester of pregnancy. Gestation progressed satisfactorily until the patient was near term, when regional lymphadenopathy became pronounced. Because of edema of the vulva, cesarean section was performed, a normal female infant weighing 6 pounds being delivered. Roentgen ray therapy was instituted following parturition, but the mother died 45 days after delivery.—A. K.

**Observations on the Nature and Treatment of Leukemia and Allied Diseases.** Lawrence, J. H. [Univ. of California, San Francisco, Calif.] *Proc. Inst. Med. Chicago*, **14**:30-49. 1942.

A preliminary discussion of the history of leukemia and its nature, etiology, and relation to dietary factors is followed by a report of investigations with radioactive isotopes. In his series of cases, the author noted a large percentage of patients with dietary inadequacy. This fact, together with the important etiological roles of benzol poisoning and heredity, is emphasized.

Since potassium after oral administration was found to be concentrated in the bone marrow and lymph nodes, leukemic patients were treated with radioactive isotopes of potassium salts. The disease appeared well controlled for a time, but later many patients seemed to become refractory to treatment. This form of therapy has the two advantages of being easily administered and not being followed by radiation reactions. Two to 4 roentgens of whole body irradiation per day are given. Polycythemia vera has been treated in this manner with considerable success, and encouraging results have been obtained in the treatment of lymphosarcomatosis. Bone sarcomas and skeletal metastases of other tumors are now being treated with the radioactive isotopes of strontium and calcium.—R. C. R.

**Primary Lymphoma.** Ledlie, R. C. B., and Smithers, D. W. *Proc. Roy. Soc. Med.*, **36**:358. 1943.

Description of a case.—E. L. K.

**Aleukemic Lymphatic Leukemia.** Matheson, A. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **14**:229-230. 1942.

A case report.—M. E. H.

**Pathology of the Genitourinary Tract in Leukemia.** Meyer, L. M. [South Nassau Communities Hosp., Rockville Center, N. Y.] *Urol. & Cutan. Rev.*, **45**:693-695. 1941.

Leukemic involvement of the kidney is of three types: there may be leukemic nodules behaving like metastases, diffuse infiltration with leukemic cells, or formation of leukemic masses in the hilum. In addition, hemorrhages are commonly found. The ureters may be diffusely invaded, but this is almost always in the late stage. Involvement of the prostate is frequent, the histological picture showing both adenomatous hyperplasia and leukemic infiltration. The seminal vesicles and testes are sometimes affected. The bladder may be involved either from the prostate, or by scattered areas of leukemic infiltration. Priapism is often described but not often found in leukemic patients. It is due either to circulatory impairment or

direct infiltration of the corpora. The author treated 2 uremic patients by renal x-radiation with good results. Irradiation of the prostate would seem advisable. Radiotherapy is contraindicated in acute cases. The author quotes Barney who found that 33% of leukemic patients coming to autopsy had genitourinary symptoms, and that 49% of all leukemic autopsies revealed some genitourinary involvement.—V. F. M.

**The Experimental Production of Leukemia and Its Significance in Relation to the Human Disease. Second Edwin R. Kretschmer Memorial Lecture. February 26, 1943.** Opie, E. L. [Cornell Univ. Med. Coll., New York, N. Y.] *Proc. Inst. Med. Chicago,* **14**:382-392. 1943.

The author reviews the experimental work that supports the conception of leukemia as a malignant disease. He separates from leukemia a variety of blood changes that bear only a superficial resemblance to it and gives a clearer understanding of their significance.—M. E. H.

**Ichthyosiform Atrophy of the Skin in Hodgkin's Disease.** Ronchese, F. [Rhode Island Hosp., Providence, R. I.] *Arch. Dermat. & Syph.,* **47**:778-781. 1943.

A report of a case in which generalized ichthyosiform atrophy of the skin became manifest in a patient with Hodgkin's disease 11 months before death. Biopsy of the skin showed atrophy and hyperkeratosis without evidence of lymphogranulomatosis. The complication is regarded as one of the many nonspecific toxic manifestations of lymphogranulomatosis.—J. G. K.

**A Survey of Hodgkin's Disease and Lymphosarcoma in Bone.** Vieta, J. O., Friedell, H. L., and Craver, L. F. [Memorial Hosp., New York, N. Y., and Univ. of California Hosp., San Francisco, Calif.] *Radiology,* **39**:1-15. 1942.

The incidence of bone involvement in Hodgkin's disease is variously reported as being 1.1 to 34%. These discrepancies result partly from differences in method and thoroughness of examination and partly from failure to differentiate between bone marrow and bone involvement.

In the present study bone lesions were demonstrated roentgenographically in 38 (14.8%) of 257 patients with Hodgkin's disease and in 15 (7%) of 213 patients with lymphosarcoma. These figures are lower than the true incidence because of the difficulty of detecting early lesions and the frequent inadequacy of the examinations. Fifty-seven per cent of the cases of Hodgkin's disease showed mixed osteolytic and osteoplastic bone lesions, while 85% of the cases of lymphosarcoma had osteolytic lesions only. The lesions in Hodgkin's disease were more numerous but progressed less rapidly than those of lymphosarcoma. The character of the lesions and the clinical course was distinctive but not pathognomonic in the two conditions. Figures are presented to show the age incidence, time of onset of bone involvement, distribution of bone lesions, and survival of patients. The 5 year survival in Hodgkin's disease was 18% and in lymphosarcoma 11%. Radiation therapy in doses of less than 2000 r usually relieved bone pain whether or not it effected an alteration in the roentgenographic appearance of the lesions. Thirty-three references are given.—C. E. D.

## Spleen

**Primary Multiple Microcystic Lymphangioma of Spleen (Case Report).** Tomlinson, W. J. [Montgomery, W. Va.] *West Virginia M. J.,* **37**:24-25. 1941.

A report of splenic lymphangiomas in a Negro male of 25 dying of carcinoma of the pancreas. The spleen was not enlarged.—E. E. S.

## Adrenal

**Sympathicoblastoma of the Right Adrenal Gland: Surgical Removal with Apparent Cure.** Hyams, J. A., and Newman, H. R. [New York Post-Graduate Med. Sch. and Hosp., New York, N. Y.] *Urol. & Cutan. Rev.,* **46**:695-696. 1942.

An 8 year old female complaining of right upper quadrant pain was found to have a sympathicoblastoma of the adrenal, adherent to the kidney. The tumor and kidney were removed after a brief course of x-radiation. The child is alive and well 3 years later.—V. F. M.

**Carcinoma of the Adrenal Cortex. Report of Four Cases.** Tuta, J. A. [Grant Hosp. and Univ. of Illinois Coll. of Med., Chicago, Ill.] *Urol. & Cutan. Rev.,* **45**:613-619. 1941.

Primary malignant adrenal cortical neoplasms are rare and much more rare than benign tumors in the cortex. Metastatic tumors to the adrenals are much more frequent than primary lesions. Females predominate in the reported cases. Four instances, 3 in males, are here recorded together with autopsy findings. Metastases had occurred to the liver and elsewhere in each patient. The differential diagnosis, particularly with reference to Cushing's syndrome and basophilic adenoma of the pituitary, is discussed. Cortical hyperplasia of the adrenals, arrhenoblastoma of the ovary, aberrant adrenal cortical tumors, thymic tumors, pineal tumors, and hereditary hirsuties also must be considered in the differential diagnosis. Other authors are cited to show that high urinary androgens are present with adrenal cortical malignant tumors, but not with Cushing's syndrome in the absence of cortical neoplasms. Surgical removal is the treatment of choice, but postoperative administration of cortical hormone, saline, sodium citrate, and a low potassium diet are also indicated.—V. F. M.

## Pancreas

**A Modified Whipple Operation for Carcinoma of the Head of the Pancreas.** Dennis, C. [Univ. of Minnesota Hosps., Minneapolis, Minn.] *Surgery,* **12**:201-206. 1942.

Case report in which end-to-end cholecystojejunostomy was performed. Regurgitation into the biliary system was minimized by the use of a long loop between this anastamosis and the Polya gastroenterostomy.—W. A. B.

**Obstructive Jaundice Due to Carcinoma of the Pancreas: The Choice of Operative Procedure.** Sallick, M. A., and Garlock, J. H. [Mt. Sinai Hosp., New York, N. Y.] *Ann. Surg.,* **115**:25-31. 1942.

In the light of personal experience the subject of obstructive jaundice due to carcinoma of the pancreas is re-

viewed and a comparison is made of the results of treatment obtained with external drainage with those of internal biliary decompression. Successful radical excision of carcinoma of the head of the pancreas is rarely possible. External biliary drainage with an operative mortality of 11.1% offers the same degree of palliation as internal biliary decompression with a mortality of 48%.—M. R. D.

**Adenoma of the Pancreas with Hyperinsulinism.** Stein, W. [Veterans' Administration, Wood, Wis.] *M. Bull. Vet. Admin.*, **18**:314-317. 1941-42.

A case report.—M. E. H.

**Radical Resection of the Pancreas for Cancer.** Todd, M. H. [St. Vincent's Hosp., Norfolk, Va.] *Virginia M. Monthly*, **68**:526-528. 1941.

A case report and the operative technic employed.—M E. H.

### Pineal

**The Conservative Treatment of Pineal Tumors.** Horrax, G., and Daniels, J. T. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North America*, **22**:649-659. 1942.

Four cases of presumed pineal tumor are reported. The lesion in these patients has not been verified pathologically, but the clinical findings and the disclosure of a characteristic shadow by ventriculography leave almost no doubt as to the diagnosis. All the patients have been treated successfully by roentgen therapy combined with a subtemporal decompression in 3 of the 4 cases. All have returned to useful life for periods of 1½ to 2½ years, with their previous symptoms almost entirely relieved.—J. L. M.

**Pinealoma. A Clinicopathologic Study of Seven Cases with a Review of the Literature.** Russell, W. O., and Sachs, E. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Arch. Path.*, **35**:869-888. 1943.

Fifty-one cases diagnosed as pinealoma are collected from the literature, to which the authors add 7. They reserve the term for the primary tumors of the pineal body that reproduce pineal tissue containing two types of cells, which frequently show a characteristic mosaic pattern. The growth occurs predominantly in males between the ages of 15 and 25 years; it arises in the pineal body and produces its symptoms by obstructing the aqueduct of Sylvius, thus causing internal hydrocephalus. Precocious puberty was observed in only 3 of the 17 patients at or below the age of 15 years; diabetes insipidus was a more frequent complication. The study indicates that pinealoma is not a functioning tumor and that it plays no direct part in the production of precocious puberty.

Pinealoma is unique in that it is an example of an autonomous new growth of a whole tissue. It may remain localized in the region of the pineal body; it may be disseminated throughout the third and lateral ventricles; or it may metastasize to the cerebral and spinal subarachnoid spaces. Because of its location and its tendency to spread, it offers little opportunity for removal. Hence the prognosis is poor. Death was known to be the final outcome in 55 of the 58 cases here collected.—J. G. K.

### Thymus

**Thymic Malignancy.** Wade, J. L., and Matthews, A. R. K. [Parkersburg, W. Va., and Rockford, Ill.] *West Virginia M. J.*, **37**:107-113. 1941.

The tumor is said to be found in 0.036 to 0.15% of autopsy examinations. The symptoms in order of frequency are: cough, dyspnea, orthopnea, hoarseness, and a visible swelling of the neck, face, or arm. The onset is often insidious. Pain is rarely present. Physical examination often fails to reveal the presence of a mass. Distention of superficial veins over the thorax, neck, and arms is a significant finding. A white female child of 5 is described as having a thymic tumor with tracheal displacement and pleural and pericardial effusion. Thymus tumors may be composed entirely of lymphocytes, or largely of reticulum cells that are derived from the epithelium of the third bronchial clefts, or of both cell types.—E. E. S.

### Thyroid

**Toxic Adenoma of the Thyroid with Hypothyroidism.** Beverly, S. S. [Veterans' Administration, Oteen, N. C.] *M. Bull. Vet. Admin.*, **18**:52-56. 1941-42.

Report of 2 cases.—M. E. H.

**Carcinoma of the Thyroid.** Smith, A. M., Jr. [Univ. of Virginia, Charlottesville, Va.] *Virginia M. Monthly*, **69**:318-324. 1942.

A review of the recent literature and a study of 26 cases led the author to the conclusions that: Clinical and pathological diagnosis of carcinoma of the thyroid is difficult; radical surgical excision followed by intensive x-ray therapy has given the best results; all nodules are potentially malignant and should be removed.—M. E. H.

**A Thyroid Tumor Affecting the Parathyroids.** Toone, W. M. [Kootenay Lake Gen. Hosp., Nelson, Canada] *Canad. M. A. J.*, **46**:271. 1942.

A case report.—A. C.

### Multiple Tumors

**Multiple Primary Malignant Neoplasms.** Asbury, M. K., and Vail, D. [Coll. of Med., Univ. of Cincinnati, Cincinnati, Ohio] *Am. J. Ophth.*, **26**:688-693. 1943.

A report of a case of malignant melanoma of the choroid and glioblastoma multiforme of the right cerebral hemisphere.—E. C. R.

**The Simultaneous Occurrence of Carcinoma and Sarcoma in the Same Uterus.** Barnes, A. C. [University Hosp., Ann Arbor, Mich.] *Am. J. Obst. & Gynec.*, **41**:135-138. 1941.

Two case reports with 28 cases collected from the literature. In the 2 new cases the growths were distinctly separate.—A. K.

**A Primary Tubal Carcinoma Associated with a Primary Ovarian Sarcoma.** Gardner, H. L., and Cain, J. C. [Univ. of Texas Sch. of Med., Galveston, Tex.] *Am. J. Obst. & Gynec.*, **42**:522-527. 1941.

A case in which 2 rare malignant tumors, a primary carcinoma of the right fallopian tube and a primary sarcoma of the right ovary, were coexistent.—A. K.

**Leiomyosarcoma of the Uterus Associated with Ramifying Angiomyoma.** Gnassi, A. M., and Price, H. P. [Jersey City Med. Center, Jersey City, N. J.] *Am. J. Obst. & Gynec.*, **42**:514-518. 1941.

A widespread angioma and a leiomyosarcomatous nodule were present within the same uterus. The authors feel that the growths represent independent processes.—A. K.

**Multiple Primary Malignant Neoplasms.** Ohlman, I. L., and Yardumian, K. Y. [Montefiore Hosp., Pittsburgh, Pa.] *Pennsylvania M. J.*, **45**:1271-1275. 1942.

Reports of 3 cases are presented: (1) Adenocarcinoma of the prostate and epithelioma of the skin. (2) Hypernephroma and adenocarcinoma of the rectum. (3) Papillary carcinoma of the bladder and adenocarcinoma of the seminal vesicles.—J. L. M.

**Theca Cell Tumor of Ovary and Endometrial Carcinoma.** Porter, J. E., and Bramhall, T. C. [Maine Gen. Hosp., Portland, Maine] *Am. J. Obst. & Gynec.*, **42**:912-914. 1941.

A case of theca cell tumor of the ovary with an associated carcinoma of the endometrium is presented. The authors feel it is reasonable to assume that the ovarian tumor played a major role in the production of the uterine tumor.—A. K.

## Miscellaneous

**The Relation of Hormones to the Development of Cancer.** Hastings, W. S. [Philadelphia, Pa.] *Pennsylvania M. J.*, **45**:960-964. 1942.

The author discusses the relationship between estrogens and the occurrence of cancer. The experimental work of Lacassagne and of Loeb in animals is reviewed. An attempt is made to answer the question concerning the extent to which conclusions from experimental work on mice carry over to human subjects.—J. L. M.

**Fibrosarcoma of the Buttock Associated with Pregnancy.** Kirtz, L. P., and Pareira, M. D. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Am. J. Obst. & Gynec.*, **42**:1066-1070. 1941.

A report of 2 cases. An unusual feature of 1 case was acute yellow atrophy, which accounted for the patient's death. It was felt that the liver condition resulted from a combination of the pregnancy and the tumor, and could not be attributed to the action of either drug or anesthetic. —A. K.

**Sacrococcygeal Tumors. Case Report.** Loe, R. H. [Seattle, Wash.] *West. J. Surg.*, **50**:202-206. 1942.

Bizarre tumors that may arise from the many embryonal and developmental defects in the sacrococcygeal region are discussed in the presentation of this case of a teratoid tumor.—M. E. H.

**Tumors of the Head, Face and Neck.** Wolfer, J. A. [Chicago, Ill.] *Indust. Med.*, **11**:528-529. 1942.

A short general discussion.—M. E. H.

## Cancer Control and Public Health

**Cancer Control in Illinois.** Brokaw, R. V. [Springfield, Ill.] *Illinois M. J.*, **82**:118-123. 1942.

The recognition that cancer is a major public health problem entails organized facilities for diagnosis and therapy, and the appreciation of the medical profession of the offered advantages.—M. E. H.

**Model Cancer Bill.** Bulletin of the American College of Radiology. *Radiology*, **39**:345-346. 1942.

The Board of Chancellors of the American College of Radiology has approved the report of a Committee appointed to study the problem of the compulsory reporting of cases of cancer.

The Committee recommended that radiologists encourage legislation to make cancer a reportable disease. This would be a public health measure designed to furnish statistical information and would discourage the practices of cancer quacks. A model bill is proposed. Cancer is now reportable in 17 states.—C. E. D.

**Reducing Mortality of Cancer.** Hix, J. B. [Altus, Okla.] *J. Oklahoma M. A.*, **34**:199-201. 1941.

Believing that much of the failure to recognize cancer in its curable stage results from superficial examination by the physician, the author urges that courses of study of the diagnosis and treatment of cancer be available for practicing physicians, and that free laboratory diagnosis of biopsy material be provided.—E. E. S.

**Cancer in Wisconsin.** Keettel, W. C. [Board of Health, Madison, Wis.] *Wisconsin M. J.*, **42**:609-610. 1943.

A request is made to physicians to achieve the original purpose of the law making cancer reportable. Efforts in this direction would not only improve cancer reporting in the State but give valuable information to gauge the weakness or effectiveness of cancer control activities.—M. E. H.

**Report of an Experiment in the Control of Cancer of the Uterus.** Macfarlane, C., Sturgis, M. C., and Fetterman, F. S. [Women's Med. Coll. of Pennsylvania, Philadelphia, Pa.] *Pennsylvania M. J.*, **45**:348-350. 1942.

In an effort to control cancer of the uterus, a group of women was secured who volunteered to come for pelvic examination twice a year for 5 years. The group was composed of 1,200 white women, 30 to 82 years of age. To date, 955 of these volunteers have been examined twice a year for 2 years. This report is based on these 3,820 examinations.

In the first 955 examinations, 4 unsuspected cancers of the uterus were found in an early and apparently curable stage. No cancers have been discovered since these. In the series of 3,820 examinations, 471 benign lesions of the pelvic organs were found. These included 291 precancerous lesions of the cervix. Treatment was recommended for 147 of these precancerous lesions and was carried out in 113 cases. The treatment consisted in cauterization (38 times), conization (15 times), surgical repair or amputation (51 times), chemical cautery (6 times), and excision of leukoplakic areas (3 times).—J. L. M.